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# JOURNAL
# OF
# DAIRY SCIENCE

VOLUME VIII

JANUARY, 1925, to NOVEMBER, 1925

1925
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

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Bernard H. Rawl

# BERNARD H. RAWL

Bernard H. Rawl, who has been for many years one of the leading figures in the dairy industry in this country, died at Berkeley, Calif., September 23, 1924.

Mr. Rawl was born at Lexington, S. C., May 2, 1876. He was graduated from the Clemson Agricultural College in 1900, and, after graduate work in dairying and animal husbandry at the Pennsylvania State College and the University of Wisconsin, he returned to Clemson as assistant in the Dairy and Animal Husbandry Department. Upon the resignation of the professor in charge Mr. Rawl became the head of the Department.

In 1905 the Dairy Division of the United States Department of Agriculture began its work on the development of dairying in the South, and Mr. Rawl was placed in charge of this work. In 1909 he was made Chief of the Dairy Division, and in 1918 he became also Assistant Chief of the Bureau of Animal Industry. He filled both of these positions until 1921 when he resigned his position in the Department to become assistant general manager of the California Central Creameries, now the Golden State Milk Products Company, the position which he held at the time of his death.

In his organization of the work in dairying in the South he showed the foresight which was one of his most striking characteristics. He recognized the rights and responsibilities of the States in the development of the dairy industries within their borders, and made it plain that as the work developed and proved profitable, the States were gradually to relieve the Federal Department of Agriculture of direct supervision and expense. His wise leadership not only accomplished this result but developed a practical system of coöperation which has greatly facilitated the progress and development of dairying in the South. In addition to the work in dairying, he conceived and aided in establishing a plan of coöperation in agricultural extension work in South Carolina by that State and the United States Department of Agriculture which eliminated duplication of work by these two agencies. This plan has since then been adopted by many, if not all, of the States.

Under Mr. Rawl's leadership as Chief the work of the Dairy Division was greatly extended. The Beltsville farm was acquired and provided with a herd, buildings, and equipment for the study of dairy herd management and problems in nutrition, milk secretion and breeding. The Experimental Creamery at Grove City, Pennsylvania, was established to provide opportunities for factory applications of methods developed in the laboratories and for the study of problems in factory management. The Western Office of the Dairy Division was established to obtain closer contact with dairying in the Western States, and dairy farming investigations were begun on the Department's farms in the semi-arid regions.

Mr. Rawl had an appreciation of the value of scientific research which is unusual in one whose daily work brought him in such close contact with the practical problems of the industry. In this appreciation he included the abstract laboratory problems as well as those relating more directly to the farm and the factory. As Chief of the Dairy Division he greatly strengthened and amplified the research work. He was especially interested in developing investigations in the nutrition and the breeding of dairy animals and the work which the Bureau of Dairying is now doing on these subjects is the direct result of his efforts. His interest in the Dairy Division did not cease when he severed his official connection with the Department. The success of the movement which resulted in the creation of a Bureau of Dairying was in a large measure due to his active interest.

Mr. Rawl's character was a mixture of strongly marked and contrasting traits. Fundamentally active and vigorous, he inspired his associates with his own high ideals of service to the public. Through his sympathetic interest in the efforts and the difficulties of others he produced about him an atmosphere of friendliness and comradeship. Outspoken in denunciation of poor work he was equally free in commendation of conscientious effort, maintaining that the one could not justly be given without the other. Thoroughly democratic in all his instincts and habits he was always ready to extend to his associates constructive and sympathetic suggestions and criticisms and to aid them in finding the places best suited to their capabilities.

While taking his duties seriously and soberly, he had an unfailing sense of humor which carried him and his fellow workers through many difficult situations. To him problems were opportunities and obstacles inspired him to renewed effort. In his work as an executive he made decisions only after careful investigation and consideration, but having decided he acted with promptness and energy. All his work was marked by keen insight, a vision of the future, and by subordination of self.

Some of the more important agricultural movements now in progress owe their inception to his efforts although his part is known only to those who have been nearest him.

In his untimely death the dairy industry has lost a leader who had a comprehensive knowledge of its problems; who saw clearly and spoke forcefully; who stood always for the strictest integrity and the highest ideals. The noble personality of Bernard H. Rawl will always remain vivid in the minds of those who knew and loved him. The influence of the wholesome and intelligent service which he rendered the dairy industry will continue long after his friends and associates have passed away.

# RESEARCH WORK OF THE BUREAU OF DAIRYING[1]

L. A. ROGERS

*In Charge, Research Laboratories, Bureau of Dairying, U. S. D. A.*

The subject assigned to me for discussion at this meeting of the Dairy Science Association is the research work in the Bureau of Dairying, but I feel that I can use your time to better advantage if I discuss the general question of research work in dairying. If I draw on the work of the Bureau for examples of how I think research work should or should not be done perhaps I will come sufficiently within the limits of my title.

The greater part of the investigational work in dairying is done with public funds and the public has a right to expect that this work will be so organized and conducted that the greatest possible returns will be secured. The public is much inclined to ignore purely scientific facts and to consider as returns from research only those advances which tend to increase the profits of industry. It behooves us, therefore, not only to obtain results of value but to coördinate our efforts so that these results will become applicable to the industry and available to the public. The organization of the research work of the Bureau of Dairying is a gradual development resulting from an attempt to meet this need.

I think I may assume that we are agreed that the progress of investigation in dairying has lagged behind that of other industries, and even behind some of the other branches of agriculture. I think you will also agree that the reason for this is the fact that in the early days research as well as instruction was left very largely to men trained in the art of dairying, but without any adequate knowledge of the basic science on which the art rests. It was inevitable that the work of these men should have been directed toward the perfection of the art rather than toward the development of a scientific basis for future progress.

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[1] Address delivered at Annual Meeting American Dairy Science Association, Milwaukee, Wisconsin, September 29, 1924.

Research workers are the product of institutions having outstanding leaders in research work and centers for dairy research are not yet sufficiently well established to develop a very large second crop of investigators.

There has been a very distinct progress, but in a large measure this has been due to the efforts of inventors rather than to organized research. One has but to consider the development of condensed and evaporated milk, milk powder and ice cream, the revolutionary changes in the butter industry brought about by the invention of the centrifugal cream separator, and the changes in machinery for handling milk and for manufacturing all types of dairy products to realize the correctness of this statement. In contrast to the work of these inventors we have some notable exceptions in men like Pasteur, Storch and Babcock whose far-reaching contributions were the result of systematic research designed for the benefit of the public.

We must admit, however, that until comparatively recent years we have been dependent for our advancement on the fortuitous results of inventors working for their personal benefit, and that many of those holding positions requiring investigation and teaching in dairying were inadequately trained in the basic sciences.

The name of the association under whose auspices we are meeting assumes that there is a science of dairying, but it would, I think, be more consistent to consider dairying as we consider medicine and agriculture in general, as an art to which many of the pure sciences are contributory. A man may be highly skilled in the art of dairying, with little knowledge of science, but a real understanding of the processes with which he is working is impossible without familiarity with the sciences of chemistry, physics, and bacteriology. Using the word SCIENCE in a broader sense we bring into our field such branches as physiology, genetics, anatomy and many others.

It is hardly necessary to urge upon this audience the necessity of the utilization of men trained in the basic sciences for investigation of the problems of the dairy industry. Many of our problems, which superficially seem relatively simple, are in reality involved problems in physical or organic chemistry, requiring a familiarity with the most advanced chemical knowledge for their solution. Uncounted generations of dairymen have watched the globules of butter appear and grow in the churn, but a physical chemist of the first rank is required to determine the steps that bring about this redistribution of the various ingredients of the cream. The coagulation of milk by heat, rennet, or acid is even more complicated, involving a simultaneous reaction in several of the highly complex constituents of the milk. When we speculate on the process by which the cow converts the raw material of the feed into the casein, albumen and lactose of the milk with the reaction and the mineral salts carefully adjusted to maintain the colloids in suspension we hardly know where to begin an investigation. Problems of this nature require for their solution the best training obtainable. They can not be solved, as some institutions have seemed to assume, by dairy chemists whose technique is confined to the Babcock test and the Westphal balance. We must go, not to the institution turning out the greatest number of successful buttermakers and herdsmen, but to those colleges and universities where research work of the highest order is done. It is only in such environment that men equal to the solution of the scientific problems of dairying will develop.

In the usual course of events an investigation which has an application to an industrial process passes through three stages. The first of these is the acquisition of basic facts without relation to their possible application. In the second stage investigators, trained in the basic science involved and familiar with the processes of the industry, apply this new information to the improvement of old methods or to the development of new products. These first two steps are essentially laboratory problems. The third stage of the investigation is the application and adaptation of the laboratory results to the field or factory routine. These steps may be completed at widely separated places and with varying time intervals. The scientific investigation may not come until the practice has been established for many generations and the results can be used only to explain what has already been developed by empirical methods. We are confronted at once with the question of the nature and the amount of work in what is usually called "pure science" which are justified in a dairy laboratory. Are we justified in using to any extent funds appropriated for investigations in dairying in carrying on problems which promise no immediate returns?

It is obvious that the basic information must be obtained by someone, somewhere. We can not build our house without a foundation and the laboratories devoted to dairy research should do their part in hewing the foundation stones. While it is futile to predict what information of value to practice may come from any fundamental investigation, the problems which a dairy laboratory should undertake may properly be limited to those sciences which have a more direct bearing on agriculture. The development of the X-ray may be traced directly to an investigation into the nature of electric discharges in high vacua. We have found the X-ray of value in studying structure of the udder and the development of eyes in Swiss cheese, but we would hardly feel justified in devoting our funds to a study of electric discharges. On the other hand, almost any chemical problem of a truly basic nature may lead to results having a direct bearing on some of our everyday problems.

Probably very few agricultural laboratories, even at the present time, would feel justified in undertaking the investigation of a problem like Pasteur's study of the tartaric acid crystals. The immediate results which he obtained, however great their interest to the chemist and crystallographer, were of no particular value to industry, but the discoveries to which this work led him have rehabilitated industries, revolutionized the practice of medicine, and founded a new science. An investigation designed to bring about these results could not have been planned at that time, because no one could have conceived the far reaching avenues opened by Pasteur's work.

While no one can predict the possible value of any investigations in pure science, nevertheless work in fundamental problems of bacteriology, in the chemistry of the fats and the proteins, the laws of heredity, or the physics of heat transmission, is almost sure to bring results that have an application to some phase of the industry.

Some of these results find their application in the most unexpected places. An extensive investigation carried on in our laboratories on the relation of hydrogen ion concentration to the growth of bacteria not only greatly facilitated the study of many other problems, but also made it possible for another investigator to perfect a method for the production of citric acid by fermentation. A study of the lag period in bacterial growth has led to what is at least a good working hypothesis for the cause of nizzler Swiss cheese.

## THE APPLICATION OF SCIENTIFIC FACTS TO PRACTICE

There is no sharp line of demarcation between what is usually called "pure science" and the application of science to practice. Any properly organized investigation of a practical problem must draw on the information in the archives of pure science and an investigation in pure science almost invariably leads to a problem in application to practice. Indeed, the greatest difficulty in attempting research in pure science in an industrial laboratory is to hold the investigator to the original problem rather than to permit him to follow the tempting leads into the field of applied science.

Investigation of industrial problems requires the same high degree of training and mental fitness which is required in the investigation of those problems which have no point of contact with commercialism.

Breeding to establish the factors governing the transmission of milk producing ability requires as great a familiarity with the established laws of heredity and as great research ability as a study of color transmission in fruit flies or guinea pigs. No one but a chemist thoroughly grounded in physical and organic chemistry should be entrusted with such a problem as the cause of deterioration of milk powder. It is not essential that an investigator of problems of this kind have previous experience in the manufacture of the product he is to study. It is necessary that he acquire a familiarity as the work progresses, but it takes much less time to make a buttermaker from a chemist than to make a chemist from a buttermaker.

Dairy problems, particularly manufacturing problems, usually involve more than one science, and in our laboratories we find that the work is greatly expedited if a team can be organized to attack the problem from several sides at once. Such a team usually consists of a chemist, a bacteriologist, and a technical man experienced in the manufacture of the particular product studied. For the latter positions there is a need of men experienced in manufacturing work or in the technical side of dairy herd management, who are also sufficiently well grounded in the basic sciences to qualify them for research work.

While a real investigator will produce results in spite of inadequate and defective equipment, progress is much more rapid in properly equipped laboratories. In an industrial laboratory the equipment should include manufacturing equipment to enable investigators to make the product on which they are working under properly controlled conditions.

In the Bureau of Dairying laboratories we are equipped for making various types of cheese, butter, ice cream, condensed and evaporated milk, milk powder, milk sugar, and other by-products. This equipment is all on a small scale, but so complete that each process can be completed exactly as in a commercial plant. This equipment is supplemented by the herd maintained on our own farm for feeding, breeding, and management investigations, which provides an ample milk supply for the operation of the experimental manufacturing plant. By means of this equipment we are able to carry on manufacturing experiments in close coöperation with what may be considered more strictly laboratory work and to do this at relatively low cost. We have been able to complete the first stages of such problems as the bacterial control of Swiss cheese, the manufacture of Roquefort cheese from cow's milk, the cause of thickening in sweetened condensed milk, and the development of tallowiness in milk powder without carrying the work to a factory.

Someone has truthfully said that a completed investigation is surrounded by interrogation points. The development of a new product suggests many questions as to its possible uses. Milk powder has possible value in baking, in milk chocolate, and in ice cream. Casein has applications too numerous to mention. Soluble albumen, a product which we have been making experimentally from whey, has been used in invalid's food, and in salad dressing.

In order that we may make a product which will be satisfactory for a particular use it is necessary to become familiar with the process in which it finds its application, and to study the properties which adapt it to that use. In problems of this kind it is obviously more satisfactory to depend on the coöperation of established laboratories rather than to attempt to extend the equipment to cover so many fields.

## APPLICATION OF LABORATORY RESULTS TO COMMERCIAL PRACTICE

When a new method has proved satisfactory under laboratory conditions, the work is by no means completed. We must first determine if it will work equally well in the field or factory, ascertain if it is profitable under average conditions, and finally convince the industry that our findings are of value.

There are numbers of reasons why methods which have worked without a flaw in the laboratory may fail when put on a commercial basis. There is first of all the probability of the introduction of factors not entering into laboratory experiments. In our laboratory manufacture we use milk produced on our own farm under better than the average conditions. Milk coming to a factory would almost certainly have a greater variety of bacterial infection, and in some phases of the work there is a probability of the feeds introducing a varying factor in the milk itself. In feeding or breeding work the limited number of animals used may give results which will not hold strictly when applied to a large number.

Perhaps most important of all is the personal factor. A new method put into commercial practice passes out of the control of its originator into the hands of operators who, perhaps, do not understand the principles involved and who may even be hostile to its introduction. The almost invariable tendency under such conditions is to attempt modifications and improvements.

A laboratory process must necessarily be modified to adapt it to varying commercial conditions, but this should be done by someone sufficiently familiar with the principles involved to avoid fundamental errors, and who is interested in correcting defects rather than in condemning the method used.

Some phases of dairy investigation are provided with opportunities for full scale experimentation in the herds and farm connected with the experiment stations, but the side concerned with the manufacture of dairy products has not usually been so fortunate.

The need for facilities for commercial scale work in dairy products has long been recognized by the Bureau of Dairying, and after two or three rather unsatisfactory attempts at coöperative arrangements, a plant was established which, while it is self-supporting, is equipped and available at all times for the manufacture on a commercial or semi-commercial scale, of all ordinary products. This serves not only for testing new methods and products, but also as a laboratory for investigations in the business of operating a creamery. Such problems as the cost of various factory operations, the transportation of milk and cream, and creamery accounting methods can be studied very readily under these conditions. Although it was never intended to be a demonstration plant, this side of its operation can not be entirely eliminated. It is very important, however, that a clear distinction be made between experimentation and demonstration. The casual visitor is very unlikely to distinguish between an experiment and a demonstration, and unfortunate reports frequently originate through this failure.

This plant serves also as a training school for men for special work in the field. In its primary function it has already rendered valuable service in the opportunity it has given for the manufacture of such cheese as Swiss, Roquefort, and Camembert on an extensive scale and for an extended period before new methods are offered to the public.

### THE DISSEMINATION OF RESULTS

When an investigator has satisfied himself that his method or product is of value he still has before him the task of convincing an incredulous public. Not only is the investigator impelled by his laudable desire to see the results of his investigation serving a useful purpose, but the public which in one or another way has paid the cost of investigation is entitled to some return on its investment. Notwithstanding all that the public has at stake in the results of research, it is slow to adopt new ideas. There is usually no difficulty in finding people with limited experience and inadequate financial resources who are willing to undertake new ventures, but the more reliable and experienced manufacturers are ordinarily willing that their competitors should do the experimenting.

The investigator in dairying may disseminate his results through the medium of special bulletins, the scientific or trade journals, the public press, the teaching force of the schools and colleges, or by demonstration.

The advantages and the limitations of department and experiment station bulletins are well known. The scientific journals reach only a limited field, but like the bulletins, serve as a permanent record. They are undoubtedly the best means of acquainting other investigators and teachers with technical details and scientific facts. The trade jounals and the public press serve to arouse interest in new projects, but are not satisfactory for complete exposition. The teachers are, very properly, conservative and pass on to their students only those principles and methods which have become established. With certain types of results a demonstration is the only satisfactory method of convincing the public of their value within a reasonable time.

The Bureau of Dairying is organized to provide for the systematic demonstration of any results which have given sufficient evidence of their commercial value. This is done through coöperation with the proper state organization and with the understanding that the assistance of the Bureau will be withdrawn whenever the project is satisfactorily established.

As an illustration of organization and methods of conducting an investigation, I may cite the work which we have done on Swiss cheese. When this work was started the Swiss cheese industry was well established in three States in this country, but the domestic cheese suffered by comparison with the imported, and the market discriminated against it. The methods of the factory did not deviate from those brought from Switzerland. The investigations in Europe were fragmentary and inconclusive and had not affected factory practice. Our investigations for a number of years were confined to the laboratory, and included chemical and bacteriological work carried on in close coôperation with small scale manufacturing. From this work there gradually evolved a method of controlling the bacterial development and the manufacturing processes which gave us under laboratory conditions a high percentage of satisfactory cheese. These methods were then put into practive on a commercial scale in the Grove City creamery, and two or three years devoted to the control of defects which developed on account of the poorer quality of the milk and the greater variety of bacterial contamination.

Coöperative arrangements have been made with two States for the demonstration of these methods, and in one State especially they have been firmly established in several factories. This work could be done at first in only one or two factories which were fortunate in having a liberal minded cheesemaker, or were unfortunate in making such a high percentage of inferior cheese that they were willing to try any remedy.

Now these factories are combined in an organization supporting a laboratory to supply cultures, and a cold storage for holding and selling their cheese. It is expected that in the near future the organization will be sufficiently well established to maintain its own field man and laboratory, and the Bureau can transfer its efforts to another field.

With any one stage of the development of this project omitted we would have now merely some printed records of an incompleted investigation and the public would have nothing to show for the many thousands of dollars expended.

Research in dairying, in common with research in nearly all industrial lines, suffers from the lack of proper organization and coördination of projects and from a deficiency of properly trained investigators. Possibly the first defect is a corollary of the second. It is encouraging, however, to observe the marked improvement which has taken place in a comparatively short time in the nature of the problems undertaken, the training and experience of the investigators and the technique of experimentation.

# THE EFFICIENCY OF UTILIZATION OF PROTEIN IN MILK PRODUCTION, AS INDICATED BY NITROGEN BALANCE EXPERIMENTS[1]

E. B. FORBES AND R. W. SWIFT

*Institute of Animal Nutrition, Pennsylvania State College, State College, Pennsylvania*

This paper is based upon data obtained by E. B. Forbes and associates in a series of 60 mineral and nitrogen balances with milch cows at the Ohio Agricultural Experiment Station, the main results having been published in Ohio bulletins Nos. 295, 308, 330 and 363, in the years 1916–1922.[2]

In these experiments the determination of the nitrogen balances was incidental to the project of investigation of the mineral metabolism, and in the above mentioned publications little use was made of the nitrogen balances, except for general confirmatory purposes in connection with the balances of the other elements studied, especially sulphur, calcium, and phosphorus.

Especial reasons for conservatism in the interpretation of this extensive series of nitrogen balances lay in the well-known adaptability of animals to different planes of protein intake, the extensive possible use of protein for energy production, and the slowness with which final effects of a particular level of protein consumption are attained.

Having refrained from attempts to use these nitrogen balances in a critical manner as the experiments have been worked up, one at a time, we have now undertaken to derive from the accumulated data average figures for efficiency of utilization of protein as indications of the proportion of the protein of the feed

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[1] Received for publication October 20, 1924.

[2] *Note:* Throughout this paper the four series of balances, conducted in different years, are designated as experiments numbered to correspond with the numbers of the bulletins cited, in which the main results of the experiments have been published.

which is recoverable in the milk. Also a comparison is made between the nutrient requirements of milk production, as observed in these experiments, and the computed requirements according to the Morrison standard.

## CONDITIONS OF EXPERIMENTATION

The balance experiments involved were conducted as a major interest, under favorable conditions, with rigorous attention to detail, and without compromise on account of the lack of any facility or attention understood to be essential to the proper conduct of the investigation.

Trained chemists were present, and usually actually engaged, in all processes which affected results. This signifies the preparation, mixing and weighing of the feeds, the feeding, care and weighing of the animals, the oversight of the collection and weighing or measuring of the excreta, and the mixing, sampling and preservation of the experimental products—the last function being performed at all times by the most experienced assistant of the laboratory staff.

If, on any account, seriously disqualifying conditions arose, the individual animals affected were immediately dropped from the experiment. There was no refused feed to be accounted for in any case.

As a general procedure all observations which could not be checked at a later date were read by two persons as they were taken and recorded.

The metabolism stalls were in a high, well-lighted and well-ventilated room in a brick building. Steam heat was available; all experiments were conducted in the winter time; and the temperature of the room was maintained, with considerable care and comparatively little departure from specification, at 50° to 55°F.

The subjects of the experiments were grade or pure-bred Holstein Friesian cows, in the prime of life, mostly in the first half of a period of lactation, though a few were at later stages. They were of the better sort ordinarily obtainable, the average daily milk production being 42.6 pounds (19.337 kgm.). In one period only was one cow bred. Cow 1, period 1, experiment 363, was in the 49th to the 68th days of pregnancy. Otherwise all of the cows were farrow.

The animals stood, or lay, upon mattresses stuffed with excelsior; were curried daily, and provided every comfort practicable under the circumstances. They could lie down and arise with freedom and at will, but no exercise outside the metabolism stalls was permissible.

In general the preliminary feeding periods were of seven to ten days, and the collection periods, from ten to twenty days duration. For the purpose of this study 45 individual period records out of the 60 available were selected, 15 being excluded on obvious accounts, such as the cows' being dry, or too far advanced in the period of lactation. The average length of the 45 experimental periods used was eighteen days.

Tables 1a, 1b and 1c comprise a statement of background data as to length of periods, live weights, weights of feeds and weights of milk produced.

In all but three of the 45 balances selected the roughage was alfalfa or clover hay, of excellent quality. In three balances the roughage was timothy hay.

The grain ration was based upon corn meal, in all cases, and in addition liberal proportions of high-class nitrogenous concen-

The mineral supplements administered were without observable effect on the milk yield, and, inasmuch as they have already been discussed at length, will receive no further mention here.

The plane of feed intake was as determined by giving the cows all that they could be depended upon to clean up absolutely without waste. This signifies that they were not quite up to full feed. When cows are eating to capacity they normally do some picking and choosing; especially they reject some of the less palatable parts of the roughage; but in these experiments there was no refused feed whatever.

As a starting point in determining the amount of feed to allow we began with a distinctly light ration, which was gradually increased toward the amount required, as indicated by prevailing rules of practice. Then as this provisional standard was ap-

TABLE 1a

*Length of experimental periods, live weights of cows, average daily rations, average amounts of milk produced, and percentages of fat in the milk*

| EXPERIMENT | PERIOD NUMBER | COW NUMBER | LENGTH OF PERIOD | LIVE WEIGHT OF COW | AVERAGE DAILY RATIONS | MILK PRODUCED | FAT IN MILK |
|---|---|---|---|---|---|---|---|
| | | | *days* | *kgm.* | *grams* | *kgm.* | *per cent* |
| 295 | I | 1 | 19 | 476 | Corn 4248; cottonseed meal 1160; timothy hay 3620; corn silage 11340; salt 23.9 | 18.405 | 3.14 |
| | | 2 | 19 | 443 | Corn 4300; cottonseed meal 1240; timothy hay 3620; corn silage 13600; salt 28 | 17.485 | 2.53 |
| | | 3 | 19 | 481 | Corn 3774; cottonseed meal 1241; timothy hay 5440; corn silage 11340; salt 23.3 | 19.854 | 2.51 |
| | | 4 | 10 | 359 | Corn 4325; cottonseed meal 818; clover hay 4080; salt 25.4 | 14.818 | 3.37 |
| | | 5 | 10 | 411 | Corn 4515; cottonseed meal 818; clover hay 4080; salt 25.4 | 14.358 | 2.76 |
| | | 6 | 10 | 352 | Corn 4310; cottonseed meal 815; clover hay 4080; salt 25.4 | 15.409 | 3.32 |
| | II | 1 | 20 | 479 | Corn 3000; cottonseed meal 1000; clover hay 4536; corn silage 11340; salt 28 | 18.462 | 3.24 |
| | | 2 | 20 | 452 | Corn 3000; cottonseed meal 1000; clover hay 4536; corn silage 11340; salt 28 | 16.485 | 2.72 |
| | | 3 | 20 | 470 | Corn 3000; cottonseed meal 1000; clover hay 4536; corn silage 11340; salt 28 | 18.605 | 2.64 |
| | | 4 | 20 | 373 | Corn 2270; distiller's grains 1730; clover hay 4082; corn silage 5444; salt 28 | 14.325 | 3.03 |
| | | 5 | 20 | 426 | Corn 2270; distiller's grains 1730; clover hay 4082; corn silage 5444; salt 28 | 14.793 | 3.20 |
| | | 6 | 20 | 371 | Corn 2270; distiller's grains 1730; clover hay 4082; corn silage 5444; salt 28 | 15.904 | 3.66 |
| | III | 1 | 20 | 475 | Corn 3000; linseed oilmeal 1250; clover hay 4536; corn silage 11340; salt 28 | 17.804 | 3.12 |
| | | 2 | 20 | 464 | Corn 3000; linseed oilmeal 1250; clover hay 4536; corn silage 14516; salt 28 | 15.229 | 2.74 |
| | | 3 | 20 | 468 | Corn 3000; linseed oilmeal 1250; clover hay 4536; corn silage 14516; salt 28 | 18.183 | 2.64 |
| | | 4 | 20 | 380 | Corn 2270; gluten feed 1720; clover hay 4536; corn silage 9072; salt 28 | 14.476 | 2.69 |
| | | 5 | 20 | 434 | Corn 2270; gluten feed 1720; clover hay 4536; corn silage 10886; salt 28 | 14.999 | 2.97 |
| | | 6 | 20 | 381 | Corn 2270; gluten feed 1720; clover hay 4536; corn silage 10886; salt 28 | 15.240 | 3.53 |

TABLE 1b

*Length of experimental periods, live weights of cows, average daily rations, average amounts of milk produced, and percentages of fat in the milk*

| EXPERIMENT | PERIOD NUMBER | COW NUMBER | LENGTH OF PERIOD | LIVE WEIGHT OF COW | AVERAGE DAILY RATIONS | MILK PRODUCED | FAT IN MILK |
|---|---|---|---|---|---|---|---|
| | | | *days* | *kgm.* | *grams* | *kgm.* | *per cent* |
| 308 | I | 1 | 20 | 532 | Corn 4092; cottonseed meal 546; wheat bran 546; linseed meal 273; corn silage 13636; clover hay 4544; salt 28.4 | 17.560 | 2.87 |
| | | 2 | 8 | 478 | Corn 3519; cottonseed meal 469; wheat bran 469; linseed meal 235; corn silage 13636; alfalfa hay 5456; salt 28 | 23.622 | 3.99 |
| | | 4 | 14 | 436 | Corn 4092; cottonseed meal 546; wheat bran 546; linseed meal 273; corn silage 11360; alfalfa hay 3636; salt 28 | 20.528 | 2.85 |
| | | 5 | 20 | 455 | Corn 4092; cottonseed meal 546; wheat bran 546; linseed meal 273; corn silage 13636; clover hay 4544; salt 28 | 18.779 | 2.77 |
| | | 6 | 20 | 508 | Corn 4092; cottonseed meal 546; wheat bran 546; linseed meal 273; corn silage 13636; clover hay 4544; salt 28 | 20.012 | 3.23 |
| | II | 1 | 20 | 520 | Corn 4092; cottonseed meal 546; wheat bran 546; linseed meal 273; corn silage 14544; clover hay 4644; salt 28; bone flour 70 | 20.224 | 3.03 |
| | | 2 | 20 | 486 | Corn 4092; cottonseed meal 546; wheat bran 546; linseed meal 273; corn silage 15908; alfalfa hay 7272; salt 56 | 26.468 | 3.27 |
| | | 3 | 20 | 395 | Corn 4432; cottonseed meal 590; wheat bran 590; linseed meal 295; corn silage 13636; alfalfa hay 3636; salt 56 | 23.117 | 2.88 |
| | | 4 | 20 | 447 | Corn 4092; cottonseed meal 546; wheat bran 546; linseed meal 273; corn silage 12728; alfalfa hay 4544; salt 56; bone flour 70 | 21.875 | 3.27 |
| | | 5 | 20 | 461 | Corn 4772; cottonseed meal 637; wheat bran 637; linseed meal 318; corn silage 15452; clover hay 4544; salt 28; calcium carbonate 70 | 20.413 | 2.69 |
| | | 6 | 20 | 515 | Corn 4092; cottonseed meal 546; wheat bran 546; linseed meal 273; corn silage 14544; clover hay 4544; salt 28; calcium carbonate 70 | 20.441 | 3.14 |

TABLE 1c

*Length of experimental periods, live weights of cows, average daily rations, average amounts of milk produced, and percentages of fat in the milk*

| EXPERIMENT | PERIOD NUMBER | COW NUMBER | LENGTH OF PERIOD | LIVE WEIGHT OF COW | AVERAGE DAILY RATIONS | MILK PRODUCED | FAT IN MILK |
|---|---|---|---|---|---|---|---|
| | | | *days* | *kgm.* | *grams* | *kgm.* | *per cent* |
| 330 | I | 1 | 20 | 440 | Corn 4989; cottonseed meal 907; linseed oilmeal 907; wheat bran 454; alfalfa hay 7256; salt 42 | 23.068 | 2.79 |
| | | 3 | 20 | 488 | Corn 4989; cottonseed meal 907; linseed oilmeal 907; wheat bran 454; alfalfa hay 7256; salt 42 | 20.244 | 2.70 |
| | | 4 | 20 | 422 | Corn 4989; cottonseed meal 907; linseed oilmeal 907; wheat bran 454; alfalfa hay 7256; salt 42; precipitated bone flour 70 | 20.841 | 3.36 |
| | | 5 | 20 | 422 | Corn 4989; cottonseed meal 907; linseed oilmeal 907; wheat bran 454; alfalfa hay 7256; salt 42; precipitated bone flour 70 | 21.642 | 2.95 |
| | | 6 | 20 | 500 | Corn 4989; cottonseed meal 907; linseed oilmeal 907; wheat bran 454; alfalfa hay 7256; salt 42; precipitate bone flour 70 | 21.261 | 2.76 |
| | II | 1 | 20 | 448 | Corn 4989; cottonseed meal 907; linseed oilmeal 907; wheat bran 454; alfalfa hay 8164; salt 42; calcium lactate 11.3 | 23.261 | 2.62 |
| | | 2 | 10 | 381 | Corn 3743; cottonseed meal 681; linseed oilmeal 681; wheat bran 340; alfalfa hay 7256; salt 42; calcium lactate 55.6 | 15.142 | 3.37 |
| | | 4 | 16 | 424 | Corn 4989; cottonseed meal 907; linseed oilmeal 907; wheat bran 454; alfalfa hay 8164; salt 42; calcium chloride 40 | 22.002 | 3.20 |
| | | 5 | 20 | 429 | Corn 4989; cottonseed meal 907; linseed oilmeal 907; wheat bran 454; alfalfa hay 8164; salt 42; calcium chloride 40 | 22.696 | 2.87 |
| | | 6 | 20 | 514 | Corn 4989; cottonseed meal 907; linseed oilmeal 907; wheat bran 454; alfalfa hay 8164; salt 42; calcium chloride 40 | 22.086 | 2.60 |
| 363 | I | 1 | 20 | 501 | Corn 5161; cottonseed meal 397; linseed oilmeal 397; wheat bran 397; alfalfa hay 7720; salt 44.5; precip. bone flour 55; precip. calcium carbonate 55 | 19.400 | 2.89 |

TABLE 1c—*Continued*

| EXPERIMENT | PERIOD NUMBER | COW NUMBER | LENGTH OF PERIOD | LIVE WEIGHT OF COW | AVERAGE DAILY RATIONS | MILK PRODUCED | FAT IN MILK |
|---|---|---|---|---|---|---|---|
| | | | *days* | *kgm.* | *grams* | *kgm.* | *per cent* |
| 363 | I | 2 | 20 | 531 | Corn 4791; cottonseed meal 369; linseed oilmeal 369; wheat bran 369; alfalfa hay 7720; salt 41.3 | 17.180 | 3.10 |
| | | 5 | 20 | 440 | Corn 4791; cottonseed meal 369; linseed oilmeal 369; wheat bran 369; alfalfa hay 7720; salt 41.3; precip. bone flour 47.1; precip. calcium carbonate 47.1 | 18.430 | 2.90 |
| | | 6 | 20 | 448 | Corn 5161; cottonseed meal 397; linseed oilmeal 397; wheat bran 397; alfalfa hay 8172; salt 44.5. | 22.505 | 3.22 |
| | III | 3 | 10 | 467 | Corn 5083; cottonseed meal 1017; wheat bran 2033; alfalfa hay 5448; salt 56.9 Fowler's solution 40. | 24.708 | 2.47 |
| | | 4 | 10 | 488 | Corn 5083; cottonseed meal 1017; wheat bran 2033; alfalfa hay 7264; salt 56.9 precip. bone flour 53.7; precip. cal. carb. 53.7; Fowler's solution 40 | 27.833 | 3.00 |

proached individual deviations were made in the amount either of grain or roughage, or of both, as necessary concessions to the preferences of the experimental subjects.

The proportion of protein in the rations was considered to be liberal. Our desire was that it be as high as could be of possible benefit as affecting the retention of the mineral nutrients.

In an effort to get the cows onto their experimental rations promptly, however, and to fix upon amounts of feed which they could be depended upon to clean up, the amounts adopted were doubtless less, in many cases, than they would have been had there been no necessity for getting onto the experimental basis with the least practicable delay.

A point to bear in mind is that the conditions of the experimental subjects in the 45 selected balances were comparable only in general terms. The plane of feed intake, the proportion of protein to non-protein nutriment, and the intimate nature of the protein combinations in the rations, were similar, but not identical. It is impossible, therefore, to treat the results as though representing ideal conditions in these regards.

Fur further details as to conditions and methods of experimentation the reader is referred to the publications already cited.

## DISCUSSION OF RESULTS

Tables 2a and 2b set forth the statistical results to be discussed. As a basis for judgment of the significance of these data an exhibit portraying the plane of nutrition will be helpful. Thus, the fourth column from the left (feed N divided by maintenance N) indicates the plane of nitrogen intake, in terms of the maintenance requirement of nitrogen.

The sufficiency of the feed intake is indicated, in relation to the nitrogen and the live-weight balances, in the two columns at the right of the table, and in relation to the Morrison feeding standard, in the third and fourth columns from the right.

In full realization of the limited significance of the individual data, we feel justified in considering that the averages of the 45 nitrogen and live weight balances have a definite value.

Of the 45 nitrogen balances 13 were negative, and 32 were positive, the average balance being +8.1 grams—thus indicating clearly that in general the nitrogen intake was sufficient to maintain equilibrium.

Of the 45 live-weight balances 24 were positive and 19 negative. In one case there was no change, and in another the data were lacking. On the average the live-weight was just maintained.

The indication, then, is that both the nitrogen and the total feed intake were, on the average, sufficient for maintenance of nutritive equilibrium during the production of 42.6 pounds of milk.

In comparison with the Morrison standard, however, the determined, apparently digestible crude protein intake was below the computed requirement in 40 of the 45 instances, the average deficiency being 0.69 pound per head; the total digestible nutrients also being below the computed requirement in 40 of the 45 instances, the average deficiency being 1.84 pounds.

TABLE 2a

*Data relating to the utilization of the nitrogen of rations for milk production. Averages per day*

| EXPERIMENT | PERIOD NUMBER | COW NUMBER | N OF FEED | FEED N ÷ MAINTENANCE N | N IN MILK | N IN BODY GAIN | UTILIZED N ÷ FEED N | UTILIZED N ÷ FEED N LESS MAINTENANCE N | DEFICIENCY OF NUTRIENTS FED: MORRISON STANDARD |  | N BALANCE | CHANGE IN LIVE WEIGHT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  |  |  | Protein | Total |  |  |
|  |  |  | gram |  | grams | grams | per cent* | per cent | pounds† | pounds‡ | grams | grams |
| 295 | I | 1 | 207.5 | 4.55 | 66.7 | +11.5 | 37.7 | 48.3 | 1.35 | 4.60 | +11.5 | −79 |
|  |  | 2 | 221.2 | 5.22 | 45.9 | +34.3 | 36.2 | 44.8 | 0.85 | −0.12 | +34.3 | +771 |
|  |  | 3 | 218.4 | 4.74 | 45.7 | +29.2 | 34.3 | 43.5 | 1.29 | 2.42 | +29.2 | −800 |
|  |  | 4 | 186.8 | 5.43 | 41.6 | +30.9 | 38.8 | 47.6 | 0.84 | 2.36 | +30.9 | 0 |
|  |  | 5 | 189.8 | 4.82 | 33.7 | +11.2 | 23.7 | 29.9 | 0.80 | 1.82 | +11.2 | +360 |
|  |  | 6 | 186.4 | 5.53 | 42.0 | +8.7 | 27.2 | 33.2 | 0.97 | 3.08 | +8.7 | −240 |
|  | II | 1 | 225.2 | 4.91 | 67.8 | +20.1 | 39.0 | 49.0 | 1.22 | 4.32 | +20.1 | −100 |
|  |  | 2 | 225.2 | 5.21 | 49.6 | +20.8 | 31.2 | 38.6 | 0.77 | 0.79 | +20.8 | +480 |
|  |  | 3 | 225.2 | 5.01 | 49.3 | +43.4 | 41.2 | 51.4 | 1.04 | 2.54 | +43.4 | +253 |
|  |  | 4 | 204.9 | 5.73 | 53.2 | +9.9 | 30.8 | 37.3 | 0.69 | 1.80 | +9.9 | −187 |
|  |  | 5 | 204.9 | 5.02 | 49.2 | +2.8 | 25.4 | 31.7 | 0.93 | 3.15 | +2.8 | +440 |
|  |  | 6 | 204.9 | 5.76 | 60.3 | +4.6 | 31.7 | 38.3 | 1.08 | 3.89 | +4.6 | −7 |
|  | III | 1 | 216.8 | 4.77 | 71.2 | +5.7 | 35.5 | 44.9 | 1.09 | 2.52 | +5.7 | +327 |
|  |  | 2 | 225.8 | 5.08 | 56.1 | +9.4 | 29.0 | 36.1 | 0.66 | −0.84 | +9.4 | +620 |
|  |  | 3 | 225.8 | 5.04 | 52.6 | +9.1 | 27.3 | 34.1 | 1.13 | 0.66 | +9.1 | −7 |
|  |  | 4 | 205.5 | 5.65 | 55.1 | +2.9 | 28.2 | 34.3 | 0.67 | −0.23 | +2.9 | +580 |
|  |  | 5 | 210.6 | 5.07 | 49.8 | +9.8 | 28.3 | 35.3 | 0.82 | 1.05 | +9.9 | +553 |
|  |  | 6 | 210.6 | 5.77 | 56.1 | +9.7 | 31.2 | 37.8 | 0.83 | 0.87 | +9.7 | +453 |
| 308 | I | 1 | 239.8 | 4.71 | 87.0 | −8.4 | 32.8 | 41.7 | 1.01 | 0.73 | −8.4 | +185 |
|  |  | 2 | 280.5 | 6.13 | 97.9 | −8.8 | 31.8 | 38.0 | 1.03 | 2.54 | −8.8 | −1827 |
|  |  | 4 | 245.8 | 5.88 | 72.3 | +7.0 | 32.3 | 38.9 | 0.87 | 2.05 | +7.0 | +849 |
|  |  | 5 | 239.8 | 5.51 | 68.2 | +6.4 | 31.1 | 38.0 | 1.14 | 0.52 | +6.4 | −473 |
|  |  | 6 | 239.8 | 4.92 | 87.0 | −5.6 | 33.9 | 42.6 | 1.50 | 3.49 | −5.6 | +11 |

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* Utilized N = milk N plus body gain or minus body loss.

† Apparently digestible crude protein, experimentally determined.

‡ Total digestible nutrients, starch-equivalent basis; experimentally determined.

At face value these data indicate that the Morrison standard calls for distinctly more protein and more total nutriment than

TABLE 2b

*Data relating to the utilization of the nitrogen of rations for milk production. Averages per day*

| EXPERIMENT | PERIOD NUMBER | COW NUMBER | N OF FEED | FEED N ÷ MAINTENANCE N | N IN MILK | N IN BODY GAIN | UTILIZED N ÷ FEED N | UTILIZED N ÷ FEED N LESS MAINTENANCE N | DEFICIENCY OF NUTRIENTS FED: MORRISON STANDARD Protein | DEFICIENCY OF NUTRIENTS FED: MORRISON STANDARD Total | N BALANCE | CHANGE IN LIVE WEIGHT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *grams* | | *grams* | *grams* | *per cent* | *per cent* | *pounds* | *pounds* | *grams* | *grams* |
| 308 | II | 1 | 253.7 | 5.09 | 82.5 | +0.8 | 32.8 | 40.8 | 1.30 | 2.94 | +0.8 | −1193 |
| | | 2 | 338.5 | 7.28 | 102.4 | −8.2 | 27.8 | 32.3 | 1.05 | 1.58 | −8.2 | +191 |
| | | 3 | 260.4 | 6.87 | 89.8 | −0.1 | 34.4 | 40.3 | 1.26 | 3.88 | −0.1 | −451 |
| | | 4 | 267.8 | 6.25 | 86.3 | +1.8 | 32.9 | 39.2 | 0.93 | 2.11 | +1.8 | −77 |
| | | 5 | 277.1 | 6.27 | 83.2 | +13.0 | 34.7 | 41.3 | 0.98 | −0.32 | +13.0 | +97 |
| | | 6 | 253.7 | 5.14 | 85.4 | +11.6 | 38.2 | 47.4 | 1.27 | 2.19 | +11.6 | +227 |
| 330 | I | 1 | 309.5 | 7.34 | 109.8 | −13.0 | 31.3 | 36.2 | 0.45 | 2.54 | −13.0 | −320 |
| | | 3 | 309.5 | 6.62 | 89.3 | −6.0 | 26.9 | 31.7 | 0.26 | 1.25 | −6.0 | −240 |
| | | 4 | 309.6 | 7.66 | 92.9 | −9.7 | 26.9 | 30.9 | 0.53 | 2.46 | −9.7 | −313 |
| | | 5 | 309.6 | 7.64 | 94.1 | −3.2 | 29.4 | 33.8 | 0.42 | 1.81 | −3.2 | +20 |
| | | 6 | 309.6 | 6.46 | 96.3 | −5.0 | 29.5 | 34.9 | 0.33 | 2.05 | −5.0 | +140 |
| | II | 1 | 386.6 | 9.01 | 113.7 | +26.2 | 36.2 | 40.7 | −0.50 | 0.20 | +26.2 | +433 |
| | | 2 | 319.0 | 8.74 | 76.5 | +20.8 | 30.5 | 34.5 | −0.73 | −0.58 | +20.8 | |
| | | 4 | 386.5 | 9.53 | 99.0 | +23.9 | 31.8 | 35.5 | −0.35 | 1.39 | +23.9 | +168 |
| | | 5 | 386.5 | 9.41 | 100.3 | +25.4 | 32.5 | 36.4 | −0.50 | 0.03 | +25.4 | +27 |
| | | 6 | 386.5 | 7.85 | 100.5 | +23.9 | 32.2 | 36.9 | −0.58 | 0.14 | +23.9 | +680 |
| 363 | I | 1 | 303.4 | 6.32 | 83.0 | +0.2 | 27.4 | 32.6 | 0.29 | 1.25 | +0.2 | −430 |
| | | 2 | 294.3 | 5.79 | 77.7 | −5.7 | 24.5 | 29.6 | 0.22 | 2.02 | −5.7 | −890 |
| | | 5 | 294.4 | 6.98 | 79.0 | −5.3 | 25.0 | 29.2 | 0.24 | 1.10 | −5.3 | +430 |
| | | 6 | 313.7 | 7.31 | 92.5 | −0.4 | 29.4 | 34.0 | 0.56 | 2.78 | −0.4 | +330 |
| | III | 3 | 315.5 | 7.06 | 97.2 | +2.2 | 31.5 | 36.7 | 0.51 | 2.82 | +2.2 | −530 |
| | | 4 | 358.2 | 7.66 | 124.4 | +6.0 | 36.4 | 41.9 | 0.72 | 5.30 | +6.0 | −380 |
| Average.......... | | | | 6.19 | | | 31.6 | 38.0 | +0.69 | +1.84 | +8.1 | +1.8 |
| Probable error of average..... | | | | | | | ±0.4 | ±0.5 | | | | |
| Probable error of single variate........ | | | | | | | ±2.8 | ±3.7 | | | | |

is required, but detracting from the basis for such an indication are the facts (1) that these cows were not bred, the nutritive requirements being less than normal on this account; (2) that the maintenance requirement of energy, under the conditions of the experiments, was probably less than normally prevails in practice; and (3) that the quality of the ration was distinctly above the average. The excessive requirements (or factors of safety) of protein and total digestible nutrients, according to the Morrison standard, therefore, were probably less than as indicated by the data—but just how much less are not matters of record.

Frankly, it is questionable whether any balance data should be considered competent, by themselves, to modify the Morrison standard, since all results obtained under laboratory conditions must be checked up under the practical conditions to which they are to apply. The suggestion, however, is that the Morrison standard is fully as high as the actual requirements.

In computing the nutritive requirement according to the Morrison standard (1) we used, in all cases, the average of the upper and the lower figures given; and in computing the feed-nutrient equivalent of the milk produced we interpolated for the nearest quarter-per cent of fat.

Columns 5 and 6, from the left, set forth the nitrogen of the milk and of the body gain or loss; and the algebraic sum of the two (which we have considered the utilized nitrogen) divided (1) by the feed nitrogen, in column 7, and (2) by the feed nitrogen minus the maintenance nitrogen, in column 8, gives us two figures, on different bases, for efficiency of utilization of the nitrogen of the ration.

The average efficiency of utilization of feed nitrogen (column 7) was 31.6 per cent, ±0.4 per cent, the standard deviation being 4.1 per cent, and the probable error of a single variate 2.8 per cent.

The average efficiency of utilization of utilizable nitrogen (feed nitrogen less maintenance nitrogen, column 8) was 38.0 per cent, ±0.5 per cent, the standard deviation being 5.4, and the probable error of a single variate 3.7 per cent.

Attention is called to the fact that the figure for nitrogen utilized covers constructive purposes only, nitrogen used for maintenance not being included, and that it is, in a sense, of mixed significance, in that it is the resultant in each case of two out of a group of three factors, the quantitative relations of which are not definitely known, to wit—(1) the nitrogen of the milk produced, (2) the nitrogen of the body gain, and (3) the nitrogen of the body loss.

In this connection it should be realized that the efficiency of utilization of the nitrogen of a particular ration may be prominently affected by the capacity of the cow to retain nitrogen as body gain, as determined by the previous plane of nitrogen intake. If the cow had been on a high plane of nitrogen intake, in the months previous to the experiment, it might be impossible for her to store any nitrogen (while in a farrow condition), no matter how generous the nitrogen intake; while, on the other hand, under other conditions as to previous feeding, she might store nitrogen rapidly and efficiently.

Whatever the conditions the nitrogen of the body gain must be considered as nitrogen utilized, and is reckoned as representing an equal amount of milk nitrogen, while the nitrogen lost from the body is considered to represent an equivalent diminution of the milk nitrogen; though we do not know accurately the quantitative relations between milk nitrogen and nitrogen of body gain or loss.

Also, if the true maintenance requirement of nitrogen is as variable, among individuals, as the maintenance requirement of energy is known to be, our measure of the plane of nitrogen intake (feed N divided by computed maintenance N) is far from a constant.

It is thus apparent that we do not have an entirely consistent and satisfactory basis for computing the amount and efficiency of the utilization of nitrogen; however, the results presented are not without value.

An effort to establish the existence of a definite relation between the plane of feed intake (as represented by the ratio of feed nitrogen to maintenance nitrogen) and the efficiency of utilization of feed nitrogen, was unsuccessful; that is, there was no observable tendency for the efficiency of utilization of nitrogen to vary regularly with the plane of intake. Assuming that there is in reality such a relationship—it seems to have been obscured, in these data, by the various existing differences in experimental conditions.

Referring to the paper of Fries, Braman and Kriss (2) the nitrogen requirement of milk production, computed in relation to the feed nitrogen, is decidedly less in the experiments here discussed, because the cows were more efficient producers, in the sense that the maintenance requirement of nitrogen was a smaller part of the nitrogen intake; but the nitrogen requirement of milk production on the feed-minus-maintenance basis was much greater in the experiments here discussed because the cows were good producers, and so were liberally fed, while the maximum efficiency of utilization of nitrogen, on this basis, would prevail only during very limited nitrogen intake and milk production. In the experiments here discussed the fact that there was, on the average, a slight nitrogen storage (8.1 grams) indicates that the nitrogen intake exceeded the requirement by amounts which we have no means of estimating, and that if less nitrogen had been fed the efficiency of utilization, on either basis, would have been somewhat higher.

## CONCLUSIONS

In a group of 45 balance periods, averaging eighteen days in length, with farrow Holstein Friesian cows in the first half of the period of lactation, producing, on the average, 42.6 pounds of milk per day, on a plane of feed intake sufficient, on the average to maintain slight nitrogen storage and live-weight equilibrium, the average utilization of feed nitrogen for milk production and body gain was 31.6 per cent, ±0.4 per cent, and the utilization of utilizable nitrogen (feed minus maintenance) was 38.0 per cent, ±0.5 per cent.

## REFERENCES

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(2) Fries, J. August, Braman, Winfred W., and Kriss, Max: On the protein requirement of milk production. Jour. Dairy Sci., vii, no. 1, 1924.

# DIFFERENT TYPES OF MILK, THEIR RELATION TO THE RENNET, AND THEIR IMPORTANCE IN CHEESEMAKING*

A. G. KOESTLER

*Federal Dairy and Bacteriological Research Station, Liebefeld, Bern, Switzerland*

According to well known scientific methods of today we are able to regulate the coagulation of milk by rennet in such a manner that a well developed coagulum is assured.

This is accomplished, first, by eliminating such milk as does not coagulate properly; second, by regulating the degree of maturity of a part of the milk to be manufactured; third, by using a pure rennet extract, well started in its lactic acid fermentation; and last, by adapting the required quantity of rennet to the peculiarities of the milk.

By carefully performing these different operations the cheesemaker is enabled to impart to his milk ability to coagulate with rennet, which guarantees to him a normal process of manufacture and a first-class product.

According to O. Hamarsten[1], the process of rennet coagulation in milk is separated into two well defined phases; first, a preparation phase, transforming the casein into a sensitive state for coagulation, and second, a coagulation phase, coagulating the para-casein complex.

The duration of the first of these two phases, the preparation phase, is limited by the time during which the milk to which rennet has been added remains liquid. The second phase begins first, with the formation of a flaky coagulum and remains in this state until the proper formation of the gel. This latter state is in a certain sense to be considered as the end phase of the phenomenon of coagulation, and includes the time from the

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* Received for publication August 1, 1924.

[1] Lehrbuch fur physiol. chemie, 1907, vi, Aufl.

first visible gelatinization of the coagulum to the more or less perfect formation of the gel. It may here be remarked that it is inherent in the phenomenon of coagulation that the several parts of its different phases are not clearly defined. The successive states of development overlap, so that at certain times very different transformation states may be observed. From the standpoint of the technique of cheesemaking, as well as from purely theoretical considerations, the structure of the gelatinization of the rennet coagulum is the especially important part of the coagulation phase in forming the curd.

In returning to more practical considerations, we find that the cheesemakers, for quite a long time, paid special attention to these phenomena of coagulation, pre-phase, coagulation, and gelatinization. The first phase, the so-called "dicklegen," or thickening of the milk, is considered to last from the time of the addition of the rennet through the "ziehen," or period of increased viscosity. After that comes coagulation proper, with its first flaky liquid stage, followed by the more or less quick transformation into the gel. The jelly thus formed then undergoes a certain number of transformations which the cheesemaker can accurately determine by means of simple tests, one of which is "Griff," or the "feel" of it. The development of the proper jelly state is very closely observed by the cheesemaker. He tries to guide the development of the different stages of the jelly in a definite direction, by means of different operations, such as turning over, cutting the curd, and heating.

Aided by long experience, the capable operator is able to separate clearly the different phases of coagulation and to study its peculiarities. These observations enable him to judge his milk and to regulate the process of manufacture.

As stated above, the cheesemaker possesses ways and means to control at least a part of the process of coagulation according to his desire. This is especially true of the so-called rennet reaction. According to the valuable researches of O. Allemann and H. Schmid[2], a certain duration of the rennet reaction equals a certain course of the coagulation phase; so that by regulation of the duration of the rennet reaction, quickness, as well as kind, of jelly formation is determined. The two named workers have shown that the intensity of jelly formation, that is, the degree of firmness of the coagulum obtained in a certain time, is inversely proportional to the duration of rennet action. It may, therefore, be assumed that in the most favorable cases of the regulation of the rennet action, the kind of jelly formation is also defined. This, of course, is limited to those cases where neither of the two phases of the rennet coagulation process already described is separately disturbed, that is, is either retarded or stimulated. Such discrepancies in the relation between duration of rennet action and kind of jelly formation may well be observed in different types of milk. It is of special importance in practical cheesemaking that these types of milk have the tendency to impart in a remarkable degree their abnormal properties to normal milk. It may sound strange to hear from cheesemakers the assertion that they can "feel" the admixture of certain tainted milks to the vat milk during cheesemaking, but such is the case, and science cannot afford to neglect much longer the elucidation of this fact. Here are fruitful facts for further investigation.

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[2] Landw. Jahrbuch der Schweiz, 1916, p. 357.

Among the problems that present themselves is the important question as to how and in what amount the adding of certain milk types to the vat milk affects the mixture in an unfavorable way. Here we are especially interested in the minor question, as to what kind of influence on the formation and texture of the gel may be expected.

These and other considerations have led us to study more closely these individual milks which do not react normally with rennet. These questions are now in course of investigation, and I refer here briefly to some of the results.

## MILK TYPE A[3]

By milk type A we understand the milk which is generally known in Switzerland as "rässalzige" milk, with a salty, pungent taste. We know today that such milk is secreted by the more or less inflamed quarters of the udder. The inflammation is usually caused by different bacteria inhabiting the udder.

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[3] According to the text we understand by this expression the more or less altered secretion of the otherwise healthy milk gland.

The different milk types are distinguished by the letters A, B, C, etc.

Chemically speaking, milk type A is characterized by its differences in composition when compared with normal milk. From a physiological standpoint the following modifications are especially noteworthy:

First, increase in the content of catalase, leucocytes, sodium chloride, highly dispersed nitrogen compounds (globulin and albumin, etc.)

Second, decrease in the content of total dry matter, fat free dry matter, milk sugar, calcium phosphate; the concentration of the hydrogen-ions and degree of acidity are decreased, and the rennet reaction is considerably diminished.

In consequence of such remarkable changes in composition as well as of alteration of a more physico-chemical nature, this secretion is distinctly abnormal in its reaction with rennet. Not only is the time of curdling shortened, but the stage of jelly formation shows a distinctly abnormal development. The peculiarities of this milk type are highly transmissible. If such milk be added to normal vat milk the possibility of manufacture of normal cheese of a high quality is seriously endangered. More extended and profound investigations will demonstrate whether this trouble is not more harmful than is now generally admitted.

Increasing the free hydrogen-ion (acidity) distinctly improves the reaction to rennet of type A. The influence of highly dispersed calcium compounds (calcium chloride) produces the same results.

How far these alterations affect the duration of the rennet reaction and the properties of the rennet gel as far as it can be externally ascertained, and the more delicate physico-chemical states of the latter, is a question that cannot be solved conclusively by simple laboratory experiment.

The results obtained in this manner are not entirely reliable under purely practical conditions. Only extended experiments in the cheese factories themselves could throw more light on this question. By increasing the rennet concentration (use of more rennet) better results are obtained, when this type of milk is used, either alone or when mixed with normal milk.

The basis for the detection of milk of this type in mixture with other milk is found in the abnormal composition of this product. In questionable cases it is of value to know that the analytical figures vary constantly in the same or approximately the same direction. The same fact can be depended on to show results when a case of slight watering of the milk might be mistaken for the trouble in question. The amount of chlorine and milk sugar present is a guide in these cases.

### MILK TYPE B[4]

In this type we meet a peculiar kind of secretion, which is as yet not well known in dairy practice. According to our observations it is not prevalent in Switzerland, where cheese of the Emmental type is manufactured. Only a cow here and there may show the trouble. It is distinguished from type A by the fact that the common analytical methods do not show any deviation from normal milk. With rennet under the usual conditions[5] no reaction takes place, and even after an increase of the hydrogen-ion concentration (acidulation) no effect is observed. If we add rennet to milk of this type in the customary way and let the mixture stand for thirty minutes, we will not be able to detect any changes, but if we now add calcium chloride to the mixture the gel is formed almost at once. This proves that while the conditions necessary for the preparation phase of the rennet reaction are present, those essential to the development of the later phases (coagulation and gelatinization) are lacking. These latter can be released only by the addition of calcium chloride.

How this type B milk originates is at present not known. Some observations not included in this paper tend to show that the cause must be inherent in the blood stream; that is, it must be active before the gland cells forming the milk are built up. It is certain that the bacterial flora of the udder has no influence on the occurrence of this trouble.

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[4] Landwirtschaftliches Jahrbuch der Schweiz, 1921, p. 330.

[5] In this experiment the usual standard rennet extract manufactured by the firm Flora at Dubendorf, Zurich, was used. The rennet concentration of the solution was such that normal milk at 35° shows the first flaky precipitate after ten to twelve minutes.

The chemistry of this milk type has been investigated by van Dam.[6] This worker supposes that such milk lacks the lime that is usually associated with the milk casein, a compound which, on account of its physico-chemical properties, is mainly responsible for the normal coagulation phase. It has already been stated that the chemical composition of this secretion is entirely normal. Especially any explanation which assumes an inflammatory state of the milk gland as the cause of milk type B, cannot be accepted. This kind of milk contains no catalase and no leucocytes. Degree of acidity as well as free hydrogen-ion content is normal. Total lime content and soluble lime (clay cell filtrate) do not give any reason for assuming that the cause of this abnormality could be found in faulty feeding, lack of lime in feedstuffs. Great irregularities in total lime content may be observed when the milk of the different quarters of the same udder is analyzed. One cow, for instance, yielded for two whole lactation periods milk of type B, except at times when a greater concentration of the secretion could be expected on physiological grounds, as for instance stoppage of milk flow caused by grave internal diseases, that is, during the acute stage of foot-and-mouth diseases, etc., and also during the period of the drying up of the cow. At such times a normal rennet reaction could be obtained, but not before or after.

In practice the type B seems not to be as dangerous as type A, because its occurrence is limited and therefore small quantities only are received at the cheese factories.

On account of its normal composition the identification of this type in a mixture with other milk is not easy. The rennet or coagulation test is not reliable except with the pure unmixed secretion.

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[6] Zeitschrift für physiologische chemie, 1908–09, lvi, 295.

### MILK TYPE C

This type is similar to type B in so far as the results of the customary chemical analysis show no deviation from the normal milk composition. With regard to its origin it resembles very much that of type B. It is only certain peculiarities in its reaction with rennet which make a separation from type B advisable at this time.

Milk type C was first detected in two cheese factories having manufacturing trouble. In these cases it was not possible to attain the necessary degree of dryness of the curd within the customary time, so that the stirring process, "ausrühren," had to be prolonged to sixty or ninety minutes or even longer. The peculiar properties of these milks were already evident at curdling time. The milk in the vat coagulated under the influence of rennet within the usual time limits; but the forming of the normal gel was considerably delayed. This delay continued throughout the entire manufacturing process and different modifications of the working methods did not bring relief.

In both cases of this peculiar trouble a relatively small addition of calcium chloride to the vat milk brought a decided improvement in the formation of the gel, especially in its end phase, so much so that the drying of the curd took place within the usual time. A further study of these phenomena conclusively demonstrated that the trouble was caused by some abnormal secretion, which, when mixed with the vat milk, influenced unfavorably its gel formation. A little later the offending milk could be isolated. At first we thought of some relation to type A, but soon the peculiar properties of this secretion made us confident that we were dealing with a new type.

With regard to origin of milk type C about the same may be said as for milk type B. Its chemical composition is similar.

As already mentioned, under the standard condition of the experiment, the duration of the rennet reaction may be entirely normal, only the gel formation is extraordinarily delayed. The distinctly curdled milk (first flaky precipitation) remains disproportionately long in its first liquid flaky phase. The evolution of the gel stage, however, is strikingly delayed.

For quite a while, a part of the milk does not share in the gel formation. This is especially to be noted and is doubtless due to the fact that the jelly remains for a relatively long time in a soft, half liquid state. The well known firm, smooth, sharply defined gel block is not formed at all, it is only after some time that we can observe a conglomerate of jelly floating in the milky whey. The contractability of the gel is also visibly lessened.

This delay in the starting and development of the gel is a distinct characteristic of milk type C. The fact that this abnormality is readily imparted to healthy milk is of great practical importance. Laboratory experiments have demonstrated that under standard condition of the experiment additions of only five to ten per cent of type C to normal milk could be readily observed by closely watching the coagulating phase, provided, of course, a strict comparison of the experiments is invariably maintained. Notwithstanding the difference in coagulation the duration of the rennet reaction in normal and tainted samples is almost identical. The unfavorable properties in the different milk samples as we discussed them, seem to be cumulative. The difference manifested itself in the kind of gel formed. When we now consider what the cheesemaker means by "Griff," or "Griffigkeit," the "feel" of it, of the curd, we can readily understand that this is a delicate method of estimating the quality of the curd. It is also evident that the capable cheesemaker, with his trained hand, is better able to detect with certainty small additions of type C to his vat milk than is the analyst with the usual laboratory methods. In laboratory experiments an increase of free hydrogen-ions had the same effect as an addition of normal milk; that is, the duration of the rennet reaction was distinctly shortened, but the quality of the gel was hardly corrected. Addition of calcium chloride to milk containing type C had about the same effect, with the difference, however, that curd formation was improved. In increase of the ferment concentration (larger amount of rennet) did not improve the quality of coagulation, nor was the duration of the rennet reaction appreciably shortened.

According to all observations to date we must pronounce milk of type C as dangerous for cheesemaking. We are sure that in addition to the factory troubles already investigated[7] more cases will eventually come to our notice, in which the disturbance in manufacturing is caused by milk type C.

The identification of this milk type C by chemical analysis is, as has been stated, difficult enough, because no externally observable abnormal condition, nor a strikingly varied composition is present. The only way of detecting this trouble is by means of the F. Schaffer rennet and coagulation test, used on a strictly comparative basis. In the use of this test it is the time rather than the kind of coagulation which should be observed. It will assist in obtaining good results, if after a certain time (for instance half normal coagulation time), the coagulated milk is tested for development and firmness of the gel. It is very important that when investigating mixed milk (patrons' milk samples) the different tests be compared closely and that completeness of coagulation (condition of whey) be considered as well as firmness of curd.

[7] Schweiz. Milchzeitung, 1923, W. 35, 36, 37, 38, 39, 40, 41, 42.

# DEVICE FOR RAPID DETERMINATION OF THE SPECIFIC GRAVITY OF CONDENSED MILK*

F. R. EVANS

*Research Laboratories, Bureau of Dairying, United States Department of Agriculture, Washington, D. C.*

One of the methods of determining the degree of concentration of sweetened condensed milk in the pan is to weigh a definite quantity of a sample on a sensitive balance. A cone-shaped copper cup holding 100 cc., designed by Mr. A. O. Dahlberg when he was a member of the department, has been used for a number of years by this laboratory for this purpose. The chief difficulty has been that when a stream of milk large enough to fill the cup rapidly was drawn, it was very hard to prevent overflowing the narrow opening, and covering the outside of the cup. This made it necessary to take time to wash and wipe the outside. If a small stream was drawn it took too long.

To overcome these difficulties a removable top has been designed to be used when filling the cup. This consists of a circular disk with a circular hole in the center just large enough to fit over the top of the cup. On the under side is a collar fitting closely around the neck of the cup and holding the upper surface of the disk even with the mouth of the cup. The joint between disk and cup is tight, but condensed milk is so thick that a ground joint is not necessary. The disk is of sufficient size to allow any milk dripping over the edge to fall clear.

When ready to take a sample, the removable top is placed upon the cup and the cup is filled direct from the pan until the milk overflows onto the disk. Then the excess milk is removed with a stroke of a spatula, the top removed, and the cup with contents weighed as usual. There may be a few drops of milk on the outside around the neck, but these are quickly removed,

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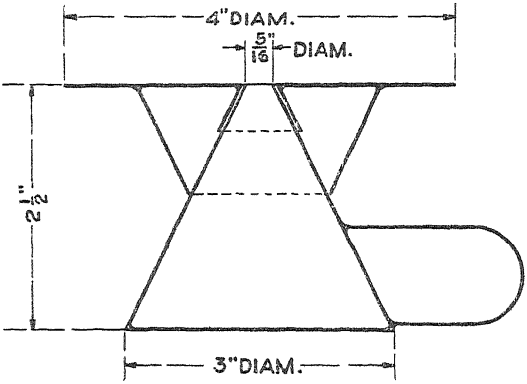
* Received for publication July 20, 1924.

whereas without the top the washing and wiping consume considerable time when quick action is essential. Variations in filling the cup with this devise ordinarily amount to not over 0.1 gram, equivalent to 0.08 degree Beaume.

The advantages of using this top are:

1. The cup may be filled more quickly.
2. The milk is prevented from running over the outside of the cup.
3. Any foam may be floated off over the top.

A diagram of a longitudinal section of the cup and top (fig. 1) is included.



LONGITUDINAL SECTION

FIG. 1

# THE SOLUBILITY OF MILK POWDER AS AFFECTED BY MOISTURE CONTENT*

G. C. SUPPLEE AND B. BELLIS

*Research Laboratory of The Dry Milk Company, Adams, New York*

Solubility is an important feature in judging the physical characteristics of milk powder. The term, "solubility" as used herein refers to the ability of desiccated milk when mixed with water to form a solution, suspension or emulsion which will simulate the physical characteristics of natural milk. The solubility of strictly fresh milk powder varies with the process of manufacture, the acidity of the liquid milk dried, and with certain conditions incident to the operation of the particular drying process involved. Another factor however, of equal, if not greater, significance in determining the degree of solubility of milk powder after a given storage period, is the question of moisture content, either that originally contained in the product or that taken up from the atmosphere.

Decreased solubility of milk powder during storage is a type of deterioration common to powders containing fat and to those made from skimmed milk. The results of studies on the deterioration of these products clearly indicate that this manifestation of deterioration is a direct result of excessive moisture, which in the great majority of cases has been found to be due to the inadequacy of the container for excluding atmospheric moisture, rather than to excessive moisture in the product at the time of manufacture. In view of the importance of this type of deterioration of milk powder, experimental data have been obtained which shows progressive changes in the solubility of the casein of milk powder as caused by varying percentages of moisture.

## EXPERIMENTAL

Since the object of this investigation was to determine the effects of original and absorbed moisture on the solubility of

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*Received for publication April 6, 1924

the proteins, comparisons were made on this constitutent only and not on the basis of the total milk solids. Fresh milk powder made by the Just double roller process was packed in air-tight containers which were so constructed that air could be circulated through the powder at will, or so that the container could be absolutely hermetically sealed as desired. Various samples all from the same original lot of powder were treated as hereinafter described. Analyses were made periodically for moisture, for the total proteins held in suspension after centrifuging, and for the casein suspended in the supernatant liquid. In order to secure uniformity of results throughout the observation period, the total solids in the original liquid milk were determined

TABLE 1

*Effect of moist air on solubility of proteins of desiccated milk (Just process)*

| AGE OF SAMPLE | PER CENT MOISTURE IN POWDER | PER CENT TOTAL PROTEIN IN SUSPENSION | PER CENT CASEIN IN SUSPENSION |
|---|---|---|---|
| Fresh | 1.95 | 2.05 | 1.88 |
| 4 days | 3.18 | 2.01 | 1.65 |
| 11 days | 5.60 | 1.80 | 1.55 |
| 18 days | 7.57 | 1.47 | 1.16 |
| 25 days | 8.15 | 1.21 | 0.98 |
| 32 days | | 0.82 | 0.68 |
| 39 days | | 0.67 | 0.46 |
| 46 days | 10.13 | 0.43 | 0.10 |
| 53 days | 11.49 | 0.19 | None |

Total protein in entire sample, 2.95 per cent; total casein in entire sample, 2.43 per cent.

TABLE 2

*Effect of moist air on solubility of proteins of desiccated milk (spray process)*

| AGE OF SAMPLE | PER CENT MOISTURE IN POWDER | PER CENT TOTAL PROTEIN IN SUSPENSION | PER CENT CASEIN IN SUSPENSION |
|---|---|---|---|
| Fresh | 1.96 | 2.27 | 1.78 |
| 7 days | 5.77 | 2.14 | 1.70 |
| 21 days | 7.25 | 1.21 | 0.92 |
| 41 days | 9.79 | 0.80 | 0.36 |
| 52 days | 10.80 | 0.25 | None |

Total protein in entire sample, 2.37 per cent; total casein in entire sample, 1.84 per cent.

and from these data the proper proportions of powder and water were mixed to obtain the same concentration of milk solids in the remade milk as were originally present in the milk before drying. The powder was mixed with water at 65°C. and held for ten minutes with constant stirring, after which a representative sample of 10 cc. was centrifuged for ten minutes at 2000 revolutions per minute on a centrifugal heat 18 cm. in diameter. The total proteins and casein were determined in the supernatant liquid which was easily decanted from the precipitate at the bottom of the tube. Tables 1 and 2 show the results obtained after circulating 1600 cc. of moist air per minute through about 300 grams of powder for a period of sixty minutes per day. The humidity of the circulated air was maintained between 75 and 80 per cent at a temperature of 75° to 80°F. During the intervals when the air was not circulated the powder was hermetically sealed. The protein and casein percentages are recorded on the basis of the original liquid milk. Since it was impossible to make analysis for the total solids in the original liquid milk dried by the spray process, the concentration ratio was determined as near as possible from the dried product. At this point it should be mentioned that the Just process powder was a partial skimmed milk product, whereas, the spray process powder was made from whole milk.

TABLE 3

*Effect of dry air on solubility of proteins of desiccated milk (Just process)*

| AGE OF SAMPLE | PER CENT MOISTURE IN POWDER | PER CENT TOTAL PROTEIN IN SUSPENSION | PER CENT CASEIN IN SUSPENSION |
|---|---|---|---|
| Fresh | 1.95 | 2.05 | 1.88 |
| 26 days | 2.33 | 2.01 | 1.80 |
| 76 days | 2.63 | 2.01 | 1.78 |
| 107 days | 2.85 | 2.00 | 1.78 |
| 140 days | 2.88 | 2.01 | 1.78 |
| 174 days | 2.88 | 2.01 | 1.78 |

Total protein in entire sample, 2.95 per cent; total casein in entire sample, 2.43 per cent.

The results given in tables 1 and 2 seem to show conclusively the direct relationship between moisture content and the development of insolubility of the casein in milk powder during storage. Regardless of the initial solubility of the powder, the rate of moisture absorption and the resulting insolubility are quite parallel in the powders made by the two different processes.

In table 3 is given the analytical results from Just process powder taken from the same batch as the powder used for the preceding determinations. This sample was handled in the same manner as the former samples, with the exception that the circulated air was first dried to a very low humidity. During the first twenty-six days there was a slight but measurable decrease in the solubility of the proteins, but thereafter, and for a period of about five months during which the dried air was continually circulated there was no measurable change in the solubility of the casein. These results are significant in showing that whatever rôle air alone (normal gases of the atmosphere with the exception of water vapor) may play in the deterioration of other constitutents of milk powder, it is not a factor in causing

TABLE 4

*Effect of moisture content of milk powder on solubility of proteins during storage —moisture 2.30 to 3.50 per cent*

| AGE OF SAMPLE | PER CENT MOISTURE IN POWDER | PER CENT TOTAL PROTEIN IN SUSPENSION | PER CENT CASEIN IN SUSPENSION |
|---|---|---|---|
| Fresh | 1.95 | 2.05 | 1.88 |
| 21 days | 2.30 | 2.05 | 1.74 |
| 42 days | 2.60 | 2.05 | 1.68 |
| 70 days | 2.65 | 2.04 | 1.70 |
| 100 days | 2.55 | 2.04 | 1.70 |
| 135 days | 2.58 | 2.04 | 1.70 |
| 175 days | 2.61 | 2.01 | 1.68 |
| 210 days | 3.01 | 2.03 | 1.74 |
| 238 days | 3.20 | 2.03 | 1.74 |
| 273 days | 3.23 | 2.04 | 1.70 |
| 298 days | 3.50 | 2.02 | 1.73 |
| 329 days | 3.45 | 1.94 | 1.70 |
| 358 days | 3.80 | 1.83 | 1.54 |
| 369 days | 7.00 | 0.35 | None |

Total protein in entire sample, 2.95 per cent; total casein in entire sample, 2.43 per cent.

insolubility of the proteins, but that this latter type of deterioration is due entirely to moisture.

The foregoing results were obtained under an artificial air and moisture environment and therefore do not permit of direct interpretation in terms of time and moisture content which bring about the development of a given degree of insolubility under normal conditions. In order to obtain further data on this particular point, the fresh powder taken from the same lot as was used for the previous tests was subjected to a treatment whereby the moisture content of three different samples was brought to a desired point immediately after manufacture. These samples were packed in containers of such a type that the moisture content could be easily controlled within one per cent for an indefinite period. Analyses were made at regular intervals for approximately one year, or until there was an absence of suspended casein after reconstituting and centrifuging as already described. In table 4 is shown the results from powder with

TABLE 5

*Effect of moisture content of milk powder on solubility of proteins during storage —moisture 4.30 to 5.20 per cent*

| AGE OF SAMPLE | PER CENT MOISTURE IN POWDER | PER CENT TOTAL PROTEIN IN SUSPENSION | PER CENT CASEIN IN SUSPENSION |
|---|---|---|---|
| Fresh | 1.95 | 2.05 | 1.88 |
| 21 days | 4.40 | 2.01 | 1.70 |
| 42 days | 4.74 | 1.92 | 1.62 |
| 70 days | 4.75 | 1.90 | 1.61 |
| 100 days | 4.70 | 1.90 | 1.60 |
| 135 days | 4.75 | 1.89 | 1.60 |
| 175 days | 4.87 | 1.90 | 1.62 |
| 210 days | 4.83 | 1.91 | 1.62 |
| 238 days | 5.13 | 1.85 | 1.49 |
| 273 days | 5.15 | 1.85 | 1.44 |
| 298 days | 5.20 | 1.79 | 1.40 |
| 329 days | 5.10 | 1.46 | 1.13 |
| 358 days | 5.15 | 1.41 | 1.13 |
| 365 days | 6.49 | 0.94 | 0.64 |
| 369 days | 6.55 | 0.89 | 0.59 |

Total protein in entire sample, 2.95 per cent; total casein in entire sample, 2.43 per cent.

moisture content maintained between 2.30 and 3.50 per cent for about one year. Similar figures are shown in table 5 for powder with a moisture content maintained between 4.30 and 5.30 per cent for the same period. Since it was desired to discontinue this work after one year and because the progress of deterioration has been clearly determined by that time the moisture content of these two samples was rapidly raised during the last few days of the observation period, with results which are entirely in harmony with those previously recorded.

TABLE 6

*Effect of moisture content of milk powder on solubility of proteins during storage —moisture approximately 5 per cent*

| AGE OF SAMPLE | PER CENT MOISTURE IN POWDER | PER CENT TOTAL PROTEIN IN SUSPENSION | PER CENT CASEIN IN SUSPENSION |
|---|---|---|---|
| Fresh | 1.95 | 2.05 | 1.88 |
| 21 days | 5.15 | 1.99 | 1.62 |
| 42 days | 5.15 | 1.87 | 1.59 |
| 70 days | 5.20 | 1.86 | 1.56 |
| 100 days | 4.95 | 1.88 | 1.58 |
| 135 days | 4.95 | 1.86 | 1.56 |
| 175 days | 4.95 | 1.86 | 1.58 |
| 210 days | 4.95 | 1.34 | 1.05 |
| 238 days | 4.85 | 1.12 | 0.69 |
| 273 days | | 0.56 | 0.16 |
| 298 days | 5.10 | 0.42 | 0.08 |
| 329 days | | 0.22 | None |

Total protein in entire sample, 2.95 per cent; total casein in entire sample. 2.43 per cent.

In table 6 is shown the results from a sample in which the moisture content was maintained as near 5 per cent as was possible. In this sample it was found that the casein had become insoluble in less than one year, and although the moisture content of this sample was not much greater than the sample shown in table 5, there was nevertheless, a marked difference in the solubility of the proteins at the end of any particular period which might be selected for comparison.

The analyses of the material precipitated by centrifuging reconstituted Just process milk powder showed from 93 to 94 per cent of the dry weight to be protein material and the remainder mineral matter. The average ash content of three samples of desiccated milk was found to be 6.846 per cent; 22.64 per cent of the ash was calcium oxide; 29.94 per cent was phosphorus pentoxide; and 2.32 per cent was magnesium oxide. The combined amounts of these constituents amounted to 54.90 per cent of the total ash. Analysis of the oven dried percipitate obtained by centrifuging the same three samples after remixing with water gave an average ash content of 7.653 per cent; 61.67 per cent of the ash was calcium oxide; 33.97 per cent was phosphorus pentoxide; and 2.65 per cent was magnesium oxide. The combined amounts of these constituents amounted to 98.29 per cent of the total ash in the sample of precipitated material. Although it is not within the scope of this paper to discuss changes in solubility of the salts of milk brought about by this method of desiccation, analysis of the precipitated mineral matter show that some of the calcium salts of the original liquid milk are altered so that their precipitation by centrifugal force is easily accomplished. It is probable that the greater part of the precipitated mineral matter is soluble calcium phosphate. These results are in accord with the observations of other investigators who have shown that a precipitation of calcium takes place during the heating and desiccation of milk.

## SUMMARY

A review of the data herein shows conclusively that the development of insolubility of proteins of milk powder during storage is due to the presence of moisture. There was a complete absence of casein in suspension after centrifuging under definite conditions at the end of seven to eight weeks during which time about 100 liters of moist air (humidity 70 to 75 per cent) were circulated daily. During this period and under the conditions of the test, the water content of the powder increased from under 2 to about 11 per cent.

The absorption of moisture and consequent development of insolubility of the casein was essentially the same in powders made by the Just double cylinder process and the spray process.

Moisture-free air circulated through milk powder at the same rate as the humid air did not cause any change in the solubility of the proteins within a period of six months, and from the results and character of the powder at the end of that time it is believed the proteins would retain their original solubility indefinitely, or as long as moisture was entirely excluded.

The original solubility of the casein of milk powder can be maintained for a year or more if the moisture content is kept below 3 per cent during the storage period. In powder with a moisture content maintained between 4 and 5 per cent there was a greater degree of insolubility throughout a period of one year than in those samples in which the moisture content was maintained at 3 or lower. Samples in which the moisture content was maintained from about 3 to about 5 per cent and in which the solubility had shown but slight change during a period of one year, became almost entirely insoluble within a few days when the moisture content was suddenly raised to 6.5 to 7 per cent. Samples in which the moisture content was maintained as near 5 per cent as possible throughout a period of one year showed greater insolubility than those samples in which the moisture content was gradually raised from 4 to 5 per cent during the same period.

The material precipitated by centrifugal force from reconstituted Just process milk powder contains approximately 93 per cent protein material and 7 per cent ash. The ash is almost entirely composed of the oxides of calcium, phosphorus and magnesium. From the analysis it appears that insoluble calcium phosphate is the principle constituent of the precipitated mineral matter.

# SOME NOTES ON THE EFFECT OF HEAT ON MEMBERS OF THE COLON TYPHOID GROUP IN MILK[1]

FRED W. TANNER AND GRANVILLE C. DUBOIS

*Department of Bacteriology, University of Illinois, Urbana, Illinois*

The possibility of members of the colon-typhoid group surviving the pasteurization process is one of ever present interest and significance. The report below is a brief outline of some experiments which bear on this problem.

## HISTORICAL

Twiss (1920) revived interest in this question when she stated that infected milk which had been heated to 60°C. and held there for a definite time and then cooled and incubated often gave growth of the organism tested. Quite a few strains of the typhoid-paratyphoid group were found to withstand 65°C. for thirty minutes in milk. Krumwiede and Noble (1921) were stimulated by the work of Twiss to test the validity of her conclusions. They stated that there was no evidence to show that members of the typhoid-paratyphoid group could endure 60°C. beyond twenty minutes. They also stated that to remove all doubt about the technic the samples of milk should be completely submerged in the bath. Krumwiede and Noble explained Twiss' results on the ground that cotton-plugged flasks were used along with other flaws in technic. It may be argued, however, that the heating of milk in closed tubes as suggested by Krumwiede and Noble does not simulate the conditions of actual pasteurization where the heating is carried out in open containers. Rosenau (1912) stated that the holding of milk at 60°C. for two minutes destroyed Bacterium typhosum. The great majority of the cells was destroyed by the time the milk reached 59°C. The dysentery bacillus was found to be a little more resistant than the typhoid bacillus. Wedemann (1919) inoculated milk with Bac-

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[1] Received for publication May 19, 1924.

terium coli communis, Bacillus suipestifer, Bacillus pyocyaneus, and the human and bovine strains of the tubercle bacillus. Biorization was found to destroy these bacteria. Ayers and Johnson (1915) noted considerable variation in the ability of 174 strains of Bacterium coli to survive pasteurization temperatures. At 60°C. 54.59 per cent of the cultures survived while at 62.8°C., 6.89 per cent survived. It was stated, however, that 62.8°C. was the critical temperature for Bacterium coli in milk and that 65.5°C. should destroy the organism. Shippen (1915) also found that Bacterium coli could survive the temperatures used in pasteurization. The thermal death point was found to be variable. In the case of Bacterium coli communior this variation was found to be as great as 13°C.

## EXPERIMENTAL

The apparatus used was that which is ordinarily found in the bacteriological laboratory. The only important special apparatus was a DeKhotinsky oil-bath containing Crisco which was used as the constant temperature device. With this it was very easy to regulate the temperature to within a fraction of a degree.

The first experiment consisted in heating milk cultures of the organisms in test tubes in an oil bath at 60°C. Two cubic centimeters of the milk cultures were delivered with sterile pipettes to the bottom of sterile test tubes 6 inches long, the greatest of care being used to prevent any milk getting onto the sides of the test tubes. These tubes were then put into racks and immersed in the oil bath to within an inch of the top of the tube. At intervals shown in table 1, loopfuls of the milk were removed and inoculated onto sterile agar slants. At the end of the experiment the contents of the test tubes were poured into sterile petri dishes with agar in order to determine whether there were any viable organism.

Examination of the data in table 1 indicates that even with a heavily infected milk, there is such a reduction in the numbers of bacteria that no viable cells were secured in loopfuls after fifteen minutes. Bacterium coli seemed to be the most resistant. However, when the milk remaining in the test tubes was poured into petri dishes with melted agar and incubated at 37°C.

for 24 hours there were a few colonies showing that a few cells survived even thirty minutes at 60°C.

The samples in this case were heavily seeded with young cells since they were twenty-four hour milk cultures. That undoubtedly accounts for the fact that a few cells were alive after thirty minutes when the whole remaining sample was tested. However, in no case using loops could viable cells be detected after fifteen minutes. These organisms were shown to give their characteristic changes on Endo's medium, Russell's double sugar agar, and lead acetate agar. This experiment was repeated with the same results with the exception that viable Bacterium coli cells could be detected for twenty minutes.

Our results in testing the remaining portion of milk in the test tube by pouring it into sterile petri dishes are in accord with those of Twiss who stated that negative tests on comparatively small amounts cannot be taken as proof of the death of all organisms.

TABLE 1

| | HEATING TIME IN MINUTES | | | | | | | | | CONTENTS OF TUBES POURED INTO DISHES 24 HOURS 37°C. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 | 20 | 25 | 30 | |
| Bacterium coli | + | + | + | + | + | + | − | − | − | + |
| Bacterium typhosum | + | + | − | − | − | − | − | − | − | − |
| Bacterium paratyphosum A. | + | + | + | − | − | − | − | − | − | − |
| Bacterium paratyphosum B. | + | + | − | − | − | − | − | − | − | − |
| Bacterium dysenteriae | + | + | + | − | − | − | − | − | − | + |

The second experiment consisted in using 0.5 cc. of a twenty-four hour broth culture of the organisms in 2 cc. of sterile milk. These tubes were heated in the oil bath at 60°C., under the same conditions as in the first experiment. The results are shown in table 2.

Comparison of the results from experiments I and II bear out the fact that the rate of death of bacteria by moist heat is a time process and depends on the number of cells which are present. In the first experiment, the number of cells was large compared to the numbers which were present in the second experiment.

There was a very satisfactory reduction from many millions to just a few colonies after thirty minutes heating at 60°C.

The third experiment consisted in heating milk which had been inoculated with 0.5 cc. of a salt suspension of the organisms. All other conditions and precautions were identical with those given under the first experiment. The results of this experiment were identical with those shown in table 2 with the exception that Bacterium coli was found to be viable for 10 minutes.

These experiments indicate that the strains were killed in much less time than thirty minutes. When twenty-four hour milk cultures were used a few viable cells were detected by pouring all of the remaining milk into petri dishes. The organisms which survived were thought to have been devitalizd since the colonies did not appear readily until the plates had been incubated for more than twenty-four hours.

TABLE 2

| | HEATING TIME IN MINUTES | | | | | | | | | | | | REMAINING MILK IN THE TEST TUBES POURED INTO STERILE TEST TUBES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5 | 7.5 | 10 | 12.5 | 15.0 | 17.5 | 20 | 22.5 | 25 | 27.5 | 30 | |
| Bacterium colon | + | + | + | + | – | – | – | – | – | – | – | – | No growth |
| Bacterium typhosum | + | + | – | – | – | – | – | – | – | – | – | – | No growth |
| Bacterium paratyphosum A | + | + | – | – | – | – | – | – | – | – | – | – | No growth |
| Bacterium paratyphosum B | + | + | – | – | – | – | – | – | – | – | – | – | No growth |
| Bacterium dysenteriae | + | + | – | – | – | – | – | – | – | – | – | – | No growth |

The fourth experiment was conducted with sealed tubes and the DeKhotinsky oil bath. The tubes used were those which were first used by Bigelow and Estey. They were 250 mm. long, 7 mm. inside diameter and 1 mm. thick. They have many advantages in the thermal death point work over the Sternberg bulb. These tubes were sterilized in the autoclav and the two sets were heated.

One set contained 2 cc. of a salt suspension of the organisms diluted about one third with sterile whole milk. The tubes were sealed off in a flame and placed in a Wassermann rack. The oil bath was regulated at 60°C. The rack holding the tubes was put into the bath and tubes removed at definite intervals. Immediately after removal these tubes were plunged into ice water to stop the action of the heat. At the end of the experiment, the contents of these tubes was poured into sterile petri dishes and plated out in agar. The results are shown in table 3. None of the strains lived beyond fifteen minutes. The milk left in the tube when poured into a sterile Petri dish, showed no living cells.

As a check on this, the same experiment was repeated with the exception that the cells were suspended in sterile salt solution. The concentration of the cells was the same as shown in table 3. *Bacterium coli* lived for less than five minutes, *Bacterium* typhosum just a little longer. None of the organisms lived for a period longer than ten minutes even though comparatively large numbers of cells were used. It is to be remembered that closed tube heating results may not be accurately compared with results collected with open tubes. In closed tubes the pressure may augment the action of the heat.

TABLE 3

*Salt suspension of cells in milk*

| | CELLS PER CUBIC CENTIMETER | HEATING TIME IN MINUTES | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 5 | 7.5 | 10 | 12.5 | 15 | 20 | 25 | 30 | 35 |
| Bacterium colon | 1,000,000 | + | + | + | + | + | − | − | − | − | − | − | − |
| Bacterium typhosum | 100,000,000 | + | + | + | + | + | − | − | − | − | − | − | − |
| Bacterium paratyphosum A | 180,000,000 | + | + | + | + | + | − | − | − | − | − | − | − |
| Bacterium paratyphosum B | 66,000,000 | + | − | + | + | − | − | − | − | − | − | − | − |
| Bacterium dysenteriae | 100,000,000 | + | + | + | + | − | − | − | − | − | − | − | − |

The last and fifth experiment was carried out after the technic of Miss Twiss except that larger flasks were used and a fresh sterile pipette used for the withdrawal of each 1 cc. specimen. One liter flasks containing 500 cc. of milk were sterilized in the autoclav. A sterile thermometer was placed in the flask and the flask heated on a hot plate to 60°C. It was then placed in the oil bath which was kept constant at 61.5 °C. The flask was placed down in the oil so that as much of its interior surface as possible was exposed to the action of the heat. Ten cubic centimeters of a twenty-four hour milk culture of the organisms used, were then introduced and carefully stirred into the milk. At intervals of one-half of a minute, 1 cc. portions were removed from the flask and plated out on dextrose agar. As stated above, each 1 cc. portion was removed with a fresh sterile pipette instead of using the same one for removing the samples. The plates were then incubated at 37°C., and the colonies counted after twenty-four to fourty-eight hours. Every attempt was made to keep the milk way below the oil line on the flask. The results are given in table 4.

Epidemiological evidence that pasteurization destroys typhoid bacilli was furnished by Geiger and Kelly (1916). The milk from a large dairy producing almost 700 gallons per day was infected by a carrier. Ninety gallons of this milk was delivered to a substation in Richmond and 600 gallons to a substation in Berkeley. The Richmond supply was not pasteurized while the Berkeley supply was. There was an epidemic of typhoid fever in Richmond traced to the milk while in Berkeley with its pasteurized supply, there was none. As Geiger and Kelly stated this shows that pasteurization destroyed the infective agent in the milk. Further epidemiological evidence is available in the experience of Chicago over a period of eleven years. As pasteurization became more common there was a general and constant decrease in the typhoid fever rate.

TABLE 4

| NAME OF ORGANISM | NUMBER OF CELLS PER CUBIC CENTIMETER | TIME REQUIRED TO DESTROY WHEN 1 CC. PORTIONS WERE TESTED |
|---|---|---|
| | | *minutes* |
| Bacterium colon | 2,000,000 | 7 |
| Bacterium typhosum | 3,500,000 | 1 |
| Bacterium paratyphosum A | 1,000,000 | 1 |
| Bacterium paratyphosum B | 500,000 | 2 |
| Bacterium dysenteriae | 1,000,000 | 1 |

This experiment was repeated twice, three times in all with concordant results.

## SUMMARY

The results of these experiments indicate that the members of the colon-typhoid group in milk in the numbers to which they would occur are destroyed by heating for thirty minutes at 60°C.

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# A COMPARATIVE STUDY OF METHODS FOR DETERMINING THE PER CENT OF BUTTERFAT IN DAIRY PRODUCTS[1]

## I. ICE CREAM

RICHARD C. FISHER AND CHARLES C. WALTS

*Storrs Agricultural Experiment Station, Storrs, Connecticut*

Recently, with the commercial introduction in this country of the Gerber method for the determination of fat in dairy products, there has arisen anew the question as to the practicability and accuracy of this method as compared with the Babcock and Mojonnier methods.

As milk and cream are now sold principally on a butterfat basis, and as standards for butterfat have been established for practically all dairy products by State and Federal Governments, the test for fat in dairy products is probably the most important and widely used test in the dairy industry. Because of this importance of the fat test to the industry and in view of the fact that relatively few comparisons between the Babcock and the Mojonnier method, and none that the authors know of on all three methods, have been made, it seemed desirable to compare the practicability and accuracy of these three tests as applied to the various dairy products.

At the time when this problem came to our attention, the most urgent need was for facts on fat tests for ice cream. Therefore, the first series of trials were made on this dairy product.

### PROCEDURE

Forty-six samples of ice cream were tested by each of the three methods. The procedure of each of these tests is outlined below.

### *Preparation of the sample*

The sample was collected by using a long butter trier or sampler reaching to the bottom of the ice cream can. Trierfuls of ice

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[1] Received for publication October 15, 1924.

cream were taken from different parts of the can and then placed in a half pint milk bottle which, after receiving the sample, was tightly stoppered with a San Lac seal cap. The sample was then melted by placing in a water bath at about 25°C. As soon as melted, the sample was thoroughly mixed and charges weighed out for each of the three methods studied.

*A. Modified Babcock.* There have been proposed numerous modifications of the Babcock Fat Test for milk and cream so as to make it applicable to ice cream. Considerable time was given over to deciding which one of these modified Babcock methods gave the best results. After making five duplicate determinations on each of five different modified Babcock tests for ice cream, the following was finally selected:

TABLE 1

*Percentages of fat in ice cream as obtained by the Mojonnier and the (alcohol-sulphuric acid) modified Babcock test*

| SAMPLE NUMBER | MOJONNIER METHOD | | MODIFIED BABCOCK METHOD | |
|---|---|---|---|---|
| | Original | Duplicate | Original | Duplicate |
| 1 | 11.31 | 11.30 | 11.20 | 11.10 |
| 2 | 13.93 | 13.91 | 12.50 | 12.50 |
| 3 | 13.23 | 13.42 | 13.00 | 13.00 |
| 4 | 12.56 | 12.50 | 12.40 | 12.30 |
| 5 | 10.31 | 10.30 | 10.00 | 10.10 |

> Weigh 9 grams of well mixed sample of ice cream into an 8 per cent milk test bottle. Add 10 cc. of 95 per cent ethyl alcohol, shake well and add 9 cc. of commercial sulphuric acid (specific gravity 1.82 to 1.83). Complete test as for milk. Multiply the results by two.

Compared with the Mojonnier and from the standpoint of clear and well defined fat column, this alcohol and sulphuric acid method gave the best results of any of the modified Babcock methods that were tried out. Table 1 shows results obtained with the alcohol and sulphuric acid test in these preliminary trials.

*B. Gerber or Troy-Fucoma method.* The Gerber fat test for milk and cream has been modified by H. C. Troy so as to make it applicable to ice cream. As the equipment is placed on the market by the Fucoma Co., the method is generally known as the Troy-Fucoma method.

The directions for making the test, as published by the Fucoma Co., were followed exactly and are briefly as follows:

1. Measure 10 cc. of diluted commercial sulphuric acid (94 cc. of acid to 6 cc. of water for chocolate ice cream or 87 cc. of acid to 13 cc. of water for other flavors) into special ice cream test bottle.
2. Weigh exactly 5 grams of well mixed sample into test bottle using care to let the ice cream run down the side of the test bottle and spread over the surface of the acid without mixing it.
3. Add 6 cc. of water and 1 cc. of amyl alcohol; insert rubber stopper and mix contents by inverting test bottles until contents are thoroughly mixed.
4. Centrifuge immediately at a speed of 1000 to 1200 revolutions per minute for about four minutes.
5. As soon as centrifuging is completed place in waterbath at 140°F. and read the fat tests by screwing the rubber stopper in or out till the bottom of the fat column is at the zero mark.

*C. Mojonnier method.* The directions for testing ice cream for fat by the Mojonnier method, as outlined in "Technical Control of Dairy Products," Mojonnier and Troy, (page 109), were followed exactly.

## RESULTS

The percentages of fat obtained on forty samples of ice cream by each of the three methods are tabulated in table 2. Thirty-eight of these samples were plain vanilla, while the two others were strawberry ice cream. An additional six samples of chocolate ice cream were also analyzed by each of the three methods. These are reported separately in table 3. To obtain a fair index as to the relative accuracy of the Troy-Fucoma and the modified Babcock methods for determining the per cent of fat in ice cream, it is necessary to compare them with a standard method of analysis, which is generally accepted as reliable and accurate. The Mojonnier or modified Roese-Gottleib method meets this requirement. Using, therefore, the Mojonnier method as the standard

TABLE 2

*Percentages of fat in ice cream as determined by the Mojonnier, the Troy-Fucoma and modified Babcock method*

| SAMPLE NUMBER* | MOJONNIER | TROY-FUCOMA | | MODIFIED BABCOCK | | PER CENT VARIATION | |
|---|---|---|---|---|---|---|---|
| | | Original | Duplicate | Original | Duplicate | Gerber from Mojonnier | Modified Babcock from Mojonnier |
| 1 | 10.65 | 11.00 | 10.90 | 10.80 | 10.80 | +0.30 | +0.15 |
| 2 | 11.47 | 11.20 | 11.00 | 11.00 | 11.10 | −0.37 | −0.42 |
| 3 | 13.97 | 13.60 | 13.50 | 14.00 | 14.10 | −0.42 | +0.08 |
| 4 | 8.13 | 8.20 | 8.40 | 8.00 | 8.05 | +0.17 | −0.10 |
| 5 | 9.77 | 10.40 | 10.40 | 9.00 | 10.00 | +0.63 | −0.27 |
| 6 | 11.65 | 11.80 | 11.90 | 11.50 | 11.40 | +0.20 | −0.20 |
| 7 | 14.43 | 14.20 | 14.00 | 13.40 | 13.50 | −0.33 | −0.98 |
| 8 | 10.58 | 9.80 | 10.00 | 9.50 | 9.60 | −0.68 | −1.03 |
| 9 | 13.62 | 13.00 | 13.20 | 13.20 | 13.00 | −0.52 | −0.52 |
| 10 | 13.73 | 13.80 | 13.90 | 13.50 | 13.40 | +0.12 | −0.28 |
| 11 | 13.37 | 13.50 | 13.70 | 13.00 | 12.90 | +0.23 | −0.42 |
| 12 | 11.31 | 11.60 | 11.40 | 11.10 | 11.20 | +0.19 | −0.16 |
| 13 | 13.23 | 13.20 | 13.40 | 13.00 | 13.00 | +0.07 | −0.23 |
| 14 | 12.56 | 12.60 | 12.60 | 12.40 | 12.30 | +0.04 | −0.21 |
| 15 | 10.31 | 10.80 | 10.90 | 10.10 | 10.10 | +0.54 | −0.26 |
| 16 | 11.87 | 11.20 | 11.20 | 11.70 | 11.60 | −0.67 | −0.22 |
| 17 | 15.47 | 15.40 | 15.40 | 15.30 | 15.20 | −0.07 | −0.22 |
| 18 | 13.71 | 14.00 | 13.80 | 13.70 | 13.51 | +0.19 | −0.11 |
| 19 | 13.31 | 12.40 | 12.60 | 13.30 | 13.20 | −0.81 | −0.06 |
| 20 | 11.54 | 12.20 | 12.00 | 11.10 | 11.20 | +0.56 | −0.39 |
| 21 | 11.83 | 10.50 | 10.40 | 11.00 | 11.00 | −1.38 | −0.83 |
| 22 | 13.91 | 13.80 | 13.80 | 13.40 | 13.50 | −0.11 | −0.46 |
| 23 | 14.15 | 13.60 | 13.80 | 14.00 | 13.90 | −0.45 | −0.20 |
| 24 | 13.44 | 13.20 | 13.20 | 13.60 | 13.40 | −0.24 | +0.06 |
| 25 | 13.15 | 13.80 | 13.90 | 13.00 | 12.80 | +0.70 | −0.24 |
| 26 | 13.38 | 14.20 | 14.00 | 13.50 | 13.40 | +0.72 | +0.07 |
| 27 | 13.36 | 13.80 | 13.80 | 12.50 | 12.50 | +0.44 | −0.86 |
| 28 | 11.11 | 10.20 | 10.10 | 11.00 | 11.50 | −0.96 | +0.14 |
| 29 | 17.05 | 17.00 | 17.00 | 17.00 | 17.00 | −0.05 | −0.05 |
| 30 | 14.07 | 15.00 | 14.80 | 14.80 | 15.00 | +0.83 | +0.83 |
| 31 | 13.67 | 14.10 | 14.20 | 13.20 | 13.20 | +0.48 | −0.47 |
| 32 | 14.39 | 13.60 | 14.00 | 14.00 | 14.00 | −0.59 | −0.39 |
| 33 | 15.66 | 15.60 | 15.80 | 15.60 | 15.60 | +0.04 | −0.06 |
| 34 | 13.66 | 13.60 | 13.80 | 14.00 | 13.80 | +0.04 | +0.24 |
| 35 | 12.55 | 12.40 | 12.20 | 12.40 | 12.30 | −0.25 | −0.20 |
| 36 | 13.44 | 13.60 | 13.40 | 13.10 | 13.00 | +0.06 | −0.39 |
| 37 | 12.95 | 12.80 | 12.80 | 13.60 | 13.40 | −0.15 | +0.55 |
| 38 | 14.42 | 15.20 | 15.00 | 15.00 | 14.90 | +0.68 | +0.53 |
| 39 | 13.57 | 13.20 | 13.00 | 13.20 | 13.30 | −0.47 | −0.32 |
| 40 | 14.72 | 14.80 | 15.00 | 14.40 | 14.50 | +0.18 | −0.27 |
| Average variation................................... | | | | | | 0.39 | 0.34 |

* All samples were vanilla ice cream except nos. 1 and 4.

for comparison, the per cent of variation of each of the Troy-Fucoma and the modified Babcock from the Mojonnier, are reported also in table 2.

A study of table 2 shows that from the standpoint of accuracy, using the Mojonnier as a standard of comparison, there is practically no difference between the Troy-Fucoma and the modified Babcock method. Thus in the case of the Troy-Fucoma 67.6 per cent of all samples checked within 0.5 per cent; while with the modified Babcock method 85 per cent of all samples checked within 0.5 per cent of the Mojonnier. A still better analysis may be made from the summary reported in table 3. The average variation from the Mojonnier on forty samples was 0.39 per cent in the case of the Troy-Fucoma and 0.34 per cent for the modified Babcock method. While this, as well as the summary in table 3 would seem to indicate slightly more favorable results for the modified Babcock, the difference is too slight to be of any significance. Moreover, when interpreting these results it must always be remembered that in studies of this type there enters in a personal factor. Before drawing final conclusions as to the practicability and accuracy of any particular method, it

TABLE 3

*Summary of percentages of variation of Troy-Fucoma from Mojonnier method*

9 samples or 28.5 per cent checked within 0.1 of 1 per cent of Mojonnier
14 samples or 37.5 per cent checked within 0.2 of 1 per cent of Mojonnier
19 samples or 47.5 per cent checked within 0.3 of 1 per cent of Mojonnier
22 samples or 55.0 per cent checked within 0.4 of 1 per cent of Mojonnier
27 samples or 67.5 per cent checked within 0.5 of 1 per cent of Mojonnier
36 samples or 90.0 per cent checked within 0.75 of 1 per cent of Mojonnier
39 samples or 97.5 per cent checked within 1.00 of 1 per cent of Mojonnier

*Summary of percentages of variation of modified Babcock from Mojonnier method*

8 samples or 20.0 per cent checked within 0.1 of 1 per cent of Mojonnier
17 samples or 42.5 per cent checked within 0.2 of 1 per cent of Mojonnier
24 samples or 60.0 per cent checked within 0.3 of 1 per cent of Mojonnier
30 samples or 75.5 per cent checked within 0.4 of 1 per cent of Mojonnier
34 samples or 85.0 per cent checked within 0.5 of 1 per cent of Mojonnier
35 samples or 87.5 per cent checked within 0.75 of 1 per cent of Mojonnier
39 samples or 97.5 per cent checked within 1.00 of 1 per cent of Mojonnier

therefore is not only desirable but necessary that comparisons such as reported in this paper be repeated in different laboratories and by several operators.

It is interesting to note that in 22 out of 40 samples, the Troy-Fucoma gave somewhat higher results than the Mojonnier. In the case of the modified Babcock only 9 samples were slightly higher while the other 31 gave results somewhat lower than the Mojonnier.

Both methods, more particularly, the Troy-Fucoma, gave uniformly clear, amber colored fat columns except for the chocolate ice cream. In the case of the chocolate ice cream, notwithstanding the special precautions as to strength and amount of acid, the fat columns in both tests frequently were indefinite or indistinct. Moreover the results obtained in practically all instances were lower than the Mojonnier.

TABLE 4

*Percentages of fat in chocolate ice cream as determined by the Mojonnier, the Troy-Fucoma and a modified Babcock method*

| SAMPLE NUMBER | MOJONNIER | TROY-EUCOMA | | MODIFIED BABCOCK | | PER CENT VARIATION | |
|---|---|---|---|---|---|---|---|
| | | Original | Duplicate | Original | Duplicate | Gerber from Mojonnier | Modified Babcock from Mojonnier |
| 1 | 10.53 | 8.60 | 8.50 | 9.80 | 8.50 | −1.98 | −1.78 |
| 2 | 9.75 | 8.00 | 8.20 | 10.40 | 10.50 | −1.65 | +0.70 |
| 3 | 14.53 | 13.20 | 13.40 | 14.00 | 14.15 | −1.23 | −0.45 |
| 4 | 11.35 | 11.20 | 11.40 | 10.50 | 10.60 | −0.05 | −0.80 |
| 5 | 12.02 | 10.80 | 11.00 | 11.00 | 11.05 | −1.12 | −0.99 |
| 6 | 13.93 | 13.50 | 13.90 | 13.50 | 13.50 | −0.08 | −0.43 |

Table 4 reports the percentages of fat in six samples of chocolate ice cream as determined by each of the three methods. While duplicate determinations of each method check closely in practically all of the six samples of chocolate ice cream, there is a large variation as compared with the Mojonnier. The nature of the chocolate or cocoa solids seemingly is such that the sulphuric acid fails to dissolve all the solids not fat or at least in some manner their properties prevent all of the fat from reaching the fat column. Judging from the uniformly low results, some of the fat is evidently held imprisoned by undissolved solids not fat or fibrous material from the cocoa.

## SUMMARY

1. In order to study the relative value of methods for determining the per cent of fat in ice cream, 46 samples were analyzed for fat by the Mojonnier, the Troy-Fucoma and a modified Babcock method.

2. Using the Mojonnier method as a standard for comparison, the results indicate that the Troy-Fucoma and the modified Babcock method used in this study rate approximately the same from the standpoint of accuracy.

3. The average per cent of variation from the Mojonnier on 40 samples was 0.39 per cent for the Troy-Fucoma and 0.34 per cent in the case of the modified Babcock method.

4. Both the Troy-Fucoma and the modified Babcock method seem well adapted for ordinary factory use where simplicity and rapidity of the test are important considerations; however, results obtained in this study would seem to make it questionable if their use is justified in instances where careful analytical results are desired.

5. It seems desirable to continue the study of these methods for determining fat in ice cream with special reference to comparing results obtained by different laboratories and operators.

# ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION[1]

## GENERAL SESSION

The nineteenth annual meeting of the American Dairy Science Association was held in connection with the National Dairy Exposition at Milwaukee, Wisconsin, September 29, 1924.

The general association meeting was held in Englemann hall, Milwaukee auditorium, Monday, September 29, 1924.

After a few brief remarks by President Borland the minutes of the 1923 meeting were read and approved. The report of the secretary-treasurer was read and adopted. A budget of $575 suggested by the Executive Committee was adopted for 1925.

The following Nominating Committee was appointed to report back to the meeting: E. S. Guthrie, chairman, L. A. Morley, O. G. Shafer, H. W. Gregory, and J. R. Dice. This committee reported the following nominations to be voted on by mail ballot before January 1, 1925: For president, O. E. Reed, H. B. Ellenberger; for vice-president, H. F. Judkins, W. M. Regan.

Under the head of divisional reports R. C. Fisher reported on the meeting of the Eastern Division and C. E. Wylie on the Southern Division meeting. No report was made of the Western Division. C. H. Eckles reported for the committee to consider affiliation with the Union of American Biological Societies. The committee recommended that the American Dairy Science Association join this Union provided the cost is not too great. Their report was adopted and the secretary instructed to notify the officers of the Biological Union of the action.

C. H. Eckles and J. A. Gamble reported on the National Research Council. A report of the meetings of the American Dairy Federation was given by O. E. Reed.

A. M. Loomis, secretary of the American Dairy Federation, was present and announced the banquet of the Federation for

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[1] These proceedings are complete except for Section III, report of which has not yet come to the Editor.

Wednesday, October 1, 1924. He also raised the question as to whether this Association would be an associate or active member in the Federation, this to be determined by the financial support given by the American Dairy Science Association.

J. H. Frandsen spoke of the JOURNAL OF DAIRY SCIENCE as did also J. A. Gamble.

A. C. Dahlberg presented a resolution on the death of Dr. Geo. A. Smith, of Geneva, New York. The resolution was adopted and a picture and statement regarding Mr. Smith will be published in the *Journal.*

The following addresses were given before a large audience: "The Research Work of the U. S. Dairy Bureau," by L. A. Rogers, U. S. Dairy Bureau. (This address appears elsewhere in this issue of the *Journal.*) "The Training and Compensation of the Research Worker," by E. D. Ball, U. S. Dept. of Agriculture.

J. H. Frandsen spoke of the possibility of creating a junior membership to the Association to include dairy students in the different colleges. C. H. Eckles mentioned the possibility of organizing local groups of the Association. After further discussion by H. A. Harding and R. C. Fisher a motion was made to appoint a committee to formulate plans and make suggestions for creating junior membership. The following committee was appointed: C. H. Eckles, chairman; J. H. Frandsen; J. B. Fitch.

As a result of a request from the International Milk Dealers Association it was moved and adopted that a committee be appointed to cooperate with the International Milk Dealers Association. H. A. Ruehe, R. C. Fisher, and G. D. Turnbow were appointed on this committee.

The question of the personnel of the Executive Committee was raised by the secretary. This was discussed by Eckles. It seemed to be the opinion that the Executive Committee, as set forth in the constitution, was sufficiently representative and less cumbersome than a larger committee.

Professor Eckles suggested that the Executive Committee consider the feasibility of having the Association meet before the opening of the Dairy Show in this way making it possible for the members to devote a day or so to the business of the Association and see the Dairy Show as well. This matter has been suggested at other times and the sentiment in favor of this arrangement is increasing. It was moved and adopted that arrangement should be made to hold the meetings of the Association either before or after the Dairy Show or at such a time that they will not conflict with the main events of the Dairy Show. The meeting adjourned at noon until 1:30 p.m. when the sectional programs were given.

### BANQUET

*September 30, 1924; 6:30 p.m.*

Toastmaster. O. E. Reed, E. Lansing, Mich.

The American Dairy Science Association, its work and purposes:

- The Production Section. J. A. Gamble, College Park, Md.
- The Manufacturing Section. H. W. Gregory, Lafayette, Ind.
- The Extension Section. L. W. Morley, State College, Penn.
- The Advanced Registry Section. Roy Harris, Madison, Wis.

The United States Dairy Bureau. C. W. Larson, Chief U. S. Dairy Bureau.

Why Dairying—in Science and in Practice? Dean H. L. Russell, University of Wis.

Announcement of Awards and Presentation of Prizes in the Inter-Collegiate Judging Contests.

### PRODUCTION SECTION

The Production Section of the American Dairy Science Association was called to order in Room-A Milwaukee Auditorium at 2 p.m., September 29, 1924, by J. A. Gamble.

Professor Gamble explained the unavoidable absence of Chairman J. J. Hooper and asked J. P. LaMaster of South Carolina to act as Secretary.

The minutes of the previous meeting were read and approved.

Prof. E. B. Hart of the University of Wisconsin gave an illustrated lecture on "Twenty Years Progress in Animal Nutrition." This address was most enthusiastically received and it was one of the most outstanding addresses of this year's meeting.

W. W. Swett of the U. S. Dairy Division reported in detail on matters relating to the student's dairy cattle judging contest. Replies from coaches showed that the time was not yet ripe for adding Brown Swiss to the breeds now used in this National contest. Report of this committee was adopted.

R. R. Graves of the U. S. Dairy Bureau gave an illustrated talk on the "Relationship of Conformation to Production in Dairy Cattle." The information was somewhat different than that contained in text books.

W. W. Swett, who during the past year has been conducting work on the "Measurements of Dairy Cattle," gave an illustrated lecture showing the variation in the measurements of heart girth, intestines and udder sections.

The election of officers of Section I resulted as follows: J. A. Gamble, University of Maryland, chairman; J. P. LaMaster, Clemson College, S. C., secretary.

### MANUFACTURE'S SECTION

The Dairy Manufacture's Section of the American Dairy Science Association was called to order by Chairman H. W. Gregory. After reading of the minutes by Secretary Fisher, Professor Hunziker reported for the Committee on Official Methods of Testing Milk and Cream for Butterfat. The following is a summary of his report. During the past year your committee collaborated with the Referee on Dairy Products of the Association of Agricultural Chemists, the Committee on Standardization of Equipment and International Association of Milk Dealers, and preliminary work was done also dealing with an investigation of the merits of the Fucoma Test and the Hoyberg Test for milk and cream.

1. Collaboration with the A. O. A. C. Your committee cooperated with Dr. Julius Hortvet, Referee on Dairy Products of the A. O. A. C. in efforts to completely revise and bring up to

date the "Official Babcock Method" of the A. O. A. C., which became obsolete. As the result of a joint meeting held in Chicago, September 14 and 15, 1923, and later conferences and correspondence, the Referee adopted for recommendation to his association the specifications for standard glassware of the A. D. S. A. and the Bureau of Standards and drafted a new set of directions for the operation of the Babcock Test. In assembling these directions, the standard directions of the A. D. S. A. served as the basis and the final draft corresponds in all fundamentals and in most of its details with our standard directions. This revised official Babcock Method, consisting of specifications for glassware and directions for operation, was subsequently adopted by the A. O. A. C. at their annual meeting in Washington, D. C., on November 19 and 20, 1923.

Slight modifications of the standard glassware were subsequently submitted for consideration and on motion favorably acted on by the A. O. A. C., and your committee was requested to submit the same for action to the A. D. S. A.; your committee explained the objections of these modifications and recommended that our Association do not favor them.

2. Collaboration with International Association of Milk Dealers. The Committee on Standardization of Equipment, R. N. Little, Secretary of the International Milk Dealers' Association, had under control the standardization of Babcock glassware for that Association. The chairman of your committee conferred with the above committee in meetings held in Chicago, December 13, 1923 and March 4, 1924, explaining the existence, history and purpose of the standard glassware adopted by our Association and by the Bureau of Standards. After full discussion, the Committee of the International Milk Dealers' Association adopted the standard glassware of our Association and recommended plans for its more general recognition and adoption by the users of the Babcock Test.

3. Investigation of Fucoma Test and Hoyberg Test. Preliminary work was undertaken to determine the suitability of these tests under American conditions. No recommendations were offered, but it was suggested that the work be continued.

The above report with its recommendations was adopted.

Dr. E. G. Hastings, in the absence of Dr. R. S. Breed reported for the Committee on Bacteriological Methods. It was pointed out by Dr. Hastings that Dr. Breed who is chairman of the committee, also acts as referee on standard methods of milk examination for the American Public Health Association. This makes possible a program of cooperation which is very essential for unified action and progress in standardizing methods of milk analysis.

Dr. Breed as part of his report, submitted copy of his report rendered as referee of the American Public Health Association. In this report he urged the need for development of:

*a.* Research methods for determining the actual number of bacteria in milk, and milk products.

*b.* Routine and verification methods for the use of public health control laboratories in controlling market milk.

*c.* Routine methods for the use of industrial concerns distributing milk and milk products.

Dr. Breed further reported slow but real progress in the efforts to standardize the composition and other ingredients of standard media. Studies have shown that particularly in the case of pasteurized milk the use of various commercial brands of peptone and beef extract bring about marked variations in the resulting bacterial counts. Efforts are therefore under way to prescribe an agar medium of such definite composition that the bacterial counts obtained from the different laboratories shall be comparable. In accordance with recommendations made by Dr. Hastings and Dr. Harding it was recommended that the methylene blue reductase test be adopted as a standard method of milk analysis.

Report of the committee was accepted.

Dr. E. S. Guthrie next presented a paper on "Trier Versus Wedge Methods of Sampling Tub Butter." Dr. Guthrie in summarizing the work stated: "No one method of sampling stands out as being the most nearly accurate. Whatever method is employed it is apparent that a creameryman who manufactures butter with less than 80.5 per cent fat or more than 15.5 per cent moisture is treading on dangerous ground." He further pointed out that there was need for further research on the problem. In the discussion that followed Professors Hunziker and Bouska brought out the practical difficulties involved in sampling cold storage butter due to the condensation of moisture.

Reporting for the Committee on Score Cards and Legal Standards, Professor J. H. Frandsen explained that although at present the committee had recommended score cards for only part of the various dairy products it had thought best to withhold further suggestions until a more complete study could be made. The committee had however devoted considerable time to a discussion of standards for ice cream and recommended the following resolution:

> WHEREAS this Association recognizes the general interest existing at this time in the question of definitions and standards for ice cream, and,
>
> WHEREAS it believes that the interest of both the consumer and the dairy industry is best served by the production of ice cream of high food value and a palatability that will tend to increase consumption:
>
> *Be it resolved* that the American Dairy Science Association does not look with favor on any movement which will result in a definition or a standard permitting the use of a low fat content or of inferior materials of any nature.

The committee explained that in drafting this resolution they had in mind to express the opinion that this Association is in favor of a high quality product and the use only of high grade materials in the manufacture of ice cream. The motion was seconded and passed.

Dr. Charles North then gave a paper on "Milk Oil as an Article of Commerce." In this paper Dr. North reviewed the history of dehydration of milk with special reference to milk fat. He pointed out that milk oil is now being made on a commercial scale and expressed the opinion that this product will soon prove of great interest to the Dairy Scientist and in the near future will compel recognition by all of the main branches of the Dairy Industry.

The next number on the program was a paper by Professor

Hunziker, on "Treatment of Cream for the Removal of Objectionable Flavors and Odors." Summarizing his report, Professor Hunziker stated that the keynote to permanent improvement in the quality of American butter lies in better cream. However, in sections where herds are small, economic conditions handicap the establishment of conditions essential to the production of cream of high quality. Cream from such sections often contains off flavors such as seasonal feed and weed flavors, flavors absorbed from foreign substances, fermentation flavor, etc. Many of these flavors are volatile or volatilizable. It is possible by intense treatment to remove most of these off flavors and thereby improve the quality of butter made from such cream. The most effective treatment according to Professor Hunziker, for removing off flavors from cream for buttermaking, consists of standardizing the cream to a low and definite point of acidity; flash pasteurizing it, and spraying it into a high vacuum, then blowing heated air through a continuous spray of the hot cream while still maintaining a partial vacuum until cream is free from all removable off flavors.

To avoid danger from premature churning and mealy texture, it is necessary to keep the cream in motion until it is cooled and to cool it rapidly without excessive agitation. Professor Hunziker pointed out that while the process has been commerically successful and is in practical operation, there is further need of research and study on such factors as: the ratio of the evaporation, the behavior of individual flavors, conditions controlling their volatilization and the physical changes of the fat units.

Dr. G. E. Holm of the Bureau of Dairying, Washington, D. C., gave an illustrated lecture on "Factors Influencing the Keeping Quality of Milk Powder." He explained the relation between the absorption of oxygen and the development of tallowness in milk fat. The speaker also pointed out that their work indicated that a milk powder with a moisture content of from two per cent down to zero is much more likely to develop talloviness than a milk powder with two and a half to three and a half per cent moisture.

The next paper presented was on "The Effect of Heat in Destroying Enzymes in Cream," by E. H. Parfitt and George

Spitzer. In their study of the effect of heat upon the inactivation of proteolytic enzymes, the holding process of pasteurization inactivated 71.07 per cent and flash pasteurization inactivated 63.57 per cent. Fresh sweet cream showed but very slight proteolytic enzyme activity while sour cream showed pronounced proteolytic enzyme activity of which 82 per cent was inactivated by holding process of pasteurization.

The evening session was called to order by Chairman Gregory. The first speaker on the program was Dr. H. A. Ruehe on "Some Factors Affecting the Quality of Ice Cream." In summarizing his paper, Dr. Ruehe stated that data accumulated thus far tends to show the following results.

1. High overruns in ice cream are more likely to cause shrinkage than low overrun.
2. Some of the so-called ripeners precipitate the casein of the ice cream mix, increasing the viscosity to the extent that under certain conditions the elasticity of the mix causes a shrinkage in the volume of the ice cream.
3. The action of the ripener does not stop with the freezing of the ice cream. An old ice cream remelted shows a flocculent curd.
4. Thawing and rehardening ice cream containing ripeners is conducive to shrinkage.

In the discussion that followed the importance of quality as a factor in the continued expansion of the ice cream industry was stressed with special reference to the quality and nature of the ingredients that go to make up the ice cream mix.

Professor J. D. Brew spoke on "Problems in Determining Milk Quality." Professor Brew urged the importance of uniformity in milk grading and milk control work and outlined how the problem has been approached in New York State and the progress made.

Professor Fisher gave a brief review of the work of the Storrs Experimental Station in regard to a "Comparative Study of Methods for Determining the Per Cent of Fat in Ice Cream" with special reference to the Fucoma method. Forty six samples of ice cream were analyzed for fat by the Mojonnier, the Fucoma and a Modified Babcock Method. The average per cent of variation from the Mojonnier was 0.39 per cent for the Fucoma and 0.34 per cent for the Modified Babcock Method. It was pointed out that the data were of a preliminary nature and that further studies are being made with special reference to the Fucoma method as to time and speed of centrifuging, temperature of reading, the fat column, and accuracy of weighing out charge on a torsion balance as compared with the chemical balance. (This paper printed in full elsewhere in this issue.—*Editor.*)

Professor W. Fisk briefly discussed the desirability of appointing a committee to make a study of the various methods for determining the per cent of fat and total solids in ice cream and moved that such a committee be appointed to report at the next annual meeting. The motion was seconded and passed. The incoming chairman later appointed the following on this committee: R. C. Fisher, chairman; H. E. Ruehe; W. Fisk.

The report of the Committee on Rules for the National Dairy Products Judging Contest, was given by Professor H. F. Judkins, chairman of the committee. The report summarized the result of a questionnaire sent to the various colleges as to the value of the contest. All are much in favor of continuing the contest.

The results of a questionnaire as to methods of financing the expenses of the teams were presented. This part of the report indicated that in most cases activities of the various dairy clubs were responsible for the expense funds.

It was decided to hold another practice ice cream judging for coaches next year, but not to include ice cream in the products to be judged by students.

Next year we go back to the old cheese score card: 45 points for flavor, 30 for body, 15 for make-up and 10 for color. The possible criticism on the various factors in scoring all products will be placed on the score card so the student will simply check the defects he finds.

Rule 14 was changed so that no Institution having a representative acting as superintendent of the butter or cheese exhibit can enter a team in the contest. A committee of two judges for each product is to be chosen in advance by the superintendent of the contest and each college is to be notified as to the membership of this committee.

Report of committee adopted.

Election of officers of Section II resulted as follows: H. F. Judkins, Amherst, Mass., chairman; N. A. Olson, Manhattan, Kans., secretary.

The meeting was then adjourned until 1925.

## OFFICIAL TESTING SECTION

The Annual Meeting of the Official Testing Section of the American Dairy Science Association was held in Milwaukee, September 29, 1924.

The meeting was presided over by R. T. Harris of the University of Wisconsin, chairman of the Section. About fifty were present representing twenty-two states; also, the secretary of the Ayrshire Breeders' Association and the president of the American Jersey Cattle Club were in attendance.

A paper sent in by A. Folger of the University of California on the Foot-and-Mouth Disease in California was read. The disease interrupted the Advanced Registry work very considerably for several weeks but through the cooperation of the breeders, the Breed Associations and the test supervisors, who took unusual precautions, the work did not seriously suffer.

M. H. Campbell of the University of Illinois described his method of handling check tests. This paper will be sent in full with the secretary's report to the several State Superintendents.

E. L. Anthony of the University of Illinois gave an interesting talk on the use of records for dairy cattle in Europe. He pointed out that about 24 per cent of all cattle in Denmark are on test during their lifetime work. Cows, to be admitted to the Record Registry, must have completed three successful yearly records.

The question of breeders in some states demanding recognition of Cow Testing Association testers was raised. In the discussion which followed it was emphasized that the primary purpose of a cow test record is to single out the good and poor workers in a herd while the Advanced Registry throws light upon the great producing and reproducing individuals. There is so much at stake in the latter records that a cow tester, in the employ of the breeders themselves, is not in a fair position to conduct Advanced Registry test for members of his testing association.

H. N. Colman of Oregon Agricultural College discussed special problems and tendencies in the Western Section. He stated that the coast states at least have divorced Cow Testing Association and Advanced Registry and that the American Dairy Science Association rules he believed were in force in all of the Rocky Mountain states.

G. C. White, chairman of the Breed Relations Committee, was then called upon for a committee report. The deliberations of this committee at its meeting the previous day at which all but one member were present and at which meeting also was Mr. Kyle of the Ayrshire Breeders' Association and Mr. and Mrs. Gardner of the Holstein-Friesian Association were briefly outlined.

The committee recommended the adoption of the revised rules for the guidance of the supervisor as presented by J. R. Dice of North Dakota Agricultural College and J. B. Fitch of Kansas Agricultural College. Slight revisions were made to clarify the rules and to have them include the actions of the Official Testing Section at its two previous meetings. With slight changes they were adopted by the Section and they follow in full below.

### *Rules for the guidance of the supervisor in the conduct of official tests*

*1. Rules must be followed.* The supervisor is not at liberty to decide as to which stipulations contained herein are essential and which are not, but is required to observe these directions in all details.

*2. Relationship of supervisor.* The supervisor shall bear in mind at all times that he is the representative of the State College or Experiment Station and the Breed Association and is not working for or taking directions from the breeder, and his duty is to see that the test is honestly made and accurately reported.

*3. Identification of animal.* The supervisor shall study the registration papers or official diagrams of each cow carefully and be sure that the cow he is testing is the animal to which the registration papers belong. Because of the difficulty of identification, each cow not having a distinctive marking must bear a tattoo mark in the ear. This mark must be recorded on the official report or the test is worthless. Unidentified cows are allowed to be placed on test, but whenever the registration papers are not at hand, a sketch must be made of the color markings and a statement made on the report as to why it was necessary to enclose the sketch. This is also necessary when the color markings do not correspond with the registration papers.

4. *Possessor considered owner.* For all purposes of Official Testing work, the possessor is considered the owner and is required to treat the animal as such, presenting all necessary data, the same as for his own.

5. *Number of milkings to be supervised at one time.* No supervisor is allowed to supervise more than twenty-four (24) milkings per day without special permission from the State Superintendent of Official Testing, unless all cows under test are milked twice daily, when a limit of thirty (30) milkings is permissible.

6. *Preliminary milkings.* All tests require a preliminary milking. In taking a preliminary milking, the supervisor shall make certain the cow is milked dry. The exact time that this milking is made should be recorded in the barn book. The last milking of the test shall be made neither earlier nor later than the time of the preliminary milking. All preliminary milkings shall be weighed, tested and reported.

7. *Rules for conducting the test:*

Before each milking period, the supervisor shall observe that the milk pail is clean and dry and shall carry the milk pail at all times during the milking period, allowing the pail out of his hands only during the actual milking process.

Only one cow shall be milked at a time, and the supervisor must in every case be in a position to observe the milker during the entire milking process.

Milking machines: Where a milking machine is used only one cow shall be milked at a time and no second man as a stripper is allowed. Milking machine is to be let run idle for a few minutes before attaching to the cow to insure that no milk is coming from another source.

Right of search: The supervisor has the right to search the milker at any time and require milkers to roll sleeves up to the elbows. Refusal on the part of the milker will be construed as evidence of intent to make a fraudulent test. In case of retest or verification test the supervisor shall search the milker.

Weighing milk: The supervisor shall weigh the empty pail before each milking. Immediately after each milking is completed he shall take charge of the pail and weigh the same on scales provided by the College and enter the exact weight of the milk at once in his barn book, and see to it that the milker records the same weight on the owner's barn milk sheet. Any inaccuracy in the owner's scales shall be reported to the superintendent in the state concerned and to the Breed Association.

Sampling milk: The supervisor shall take a sample of the milk of each milking immediately upon the weighing of the milk, being careful that the milk is thoroughly mixed. Such samples shall be kept under lock and key or in the supervisor's sight until the samples have cooled to ordinary room temperature, which is 60° to 70°F. When the last samples at night are kept until morning, unless thoroughly cooled in warm weather, preservative should be added to each sample. These cooled samples shall be warmed to 70°F. before samples are taken for testing.

Applying the Babcock test: The fat determination shall always be made in duplicate, and both determinations recorded. The average must be used in computing the amount of fat. Readings of the tests shall be made at a temperature of 130–140°F. This temperature to be obtained by placing the bottles in a hot water bath for 5 minutes. If the duplicate determinations vary more than 0.2 of one per cent the test must be repeated. The samples taken at any one milking shall not be thrown away until satisfactory duplicate tests of the milking are obtained.

*8. Lost milk and lost samples.* No substitution of lost milkings or lost samples is allowed. Any missing data due to loss of milk weights or test samples are to be left blank on the report.

*9. Preliminary reports.* The supervisor shall report to the Superintendent of Official Testing any obvious violations of the rules of the College or the Breed Associations on the part of the owner or attendants, and shall report immediately any cows that meet retest requirements and note upon his report form any sickness of a cow or other condition likely to affect the reliability of the test.

A preliminary report card shall be mailed by the supervisor from the farm to the Breed Association immediately after the test. Any cow not in normal condition should be reported on this card.

*10. Reports.* The supervisor shall make out his reports so as to give all of the information asked for and then sign and mail them promptly to his appointing officer for checking and endorsement.

*11. Test periods exceeding two days.* Where a test is conducted longer than the regular two days, or where more than one test is made in a month, all details of each test from beginning to end shall be reported.

*12. Payment of supervisors.* Under no circumstances shall any payment, gift or gratuity to the supervisor be made by, or permitted from the owner of the cow or anyone interested in her, and any violation of this rule will invalidate the test.

*13. Supervisors to supervise tests.* The supervisor appointed to conduct a test is the direct representative of the Superintendent of Official Testing and should be respected accordingly. His duties are to supervise tests only.

*14. Sworn statements.* Each supervisor is required to fill out a sworn statement covering all his work, which eliminates the necessity of his affirming test reports.

*15. Responsibility for enforcement of rules.* Owners and persons in their employ are held equally responsible with the supervisor for the enforcement of the foregoing rules. Test reports will not be accepted unless all of the above rules have been observed by all parties concerned.

G. E. Morton of Colorado Agricultural College spoke about having the supervisor take oath. It was pointed out that the common practise in most states is to have the supervisor take oath when he takes up his work or as in other states to take oath once each month covering all the work for that month.

Chairman White then reported on the meeting of a sub-committee of the Breed Relations Committee which had been instructed to interview the American Jersey Cattle Club with reference to the enforcement of the preliminary milking rule. O. E. Reed of Michigan Agricultural College, R. T. Harris of the University of Wisconsin, C. E. Wylie of the University of Tennessee and G. E. White of the Connecticut Agricultural College comprised the committee. This committee had a session with Mr. M. D. Munn and Mr. O. H. Baker of the American Jersey Cattle Club.

Mr. Munn assured the committee that they wanted to place all necessary safeguards around their tests. The committee told him there seemed to be sufficient evidence now on the value of the preliminary milking: for setting a time of beginning the test to prevent or discourage padding milk weights; and to prevent or discourage leaving strippings in the udder which both raises the test and the milk yield during the test. Mr. Baker stated that they could detect this practise with their checking system. The committee then proposed that the Jersey Club should take the entire responsibility for this matter and preliminary milkings on Jerseys should be discontinued everywhere. Messrs. Munn and Baker then stated that the whole matter would be placed before their Board and that they would make every effort to come to a satisfactory settlement of the whole matter at an early date.

After considerable discussion, the Breed Relations Committee was authorized to continue negotiations with the American Jersey Club until a satisfactory settlement is reached, the matter to be reported back at the next meeting.

Election of officers of Section IV for the ensuing year resulted in the selection of G. C. White, Storrs, Conn., chairman; C. E. Wylie, Knoxville, Tenn., secretary.

The following committees have been appointed by the chairman:

*Breed Relations Committee:* W. M. Regan, Calif.; J. B. Fitch, Kans.; A. A. Borland, Penn.; J. R. Dice, N. D.; M. H. Campbell, Ill.; O. E. Reed, Mich.; C. E. Wylie, Tenn., chairman.

*Investigation Committee:* R. T. Harris, Wis., chairman; H. N. Colman, Ore.; J. S. Peterson, Minn.; A. R. Darnell, Texas.

Signed: G. C. White,
*Secretary.*

## AWARDS OF CUPS AND MEDALS

### Students Contest in Judging Dairy Products

### NATIONAL DAIRY EXPOSITION, MILWAUKEE, WIS., 1924

| | |
|---|---|
| National Dairy Exposition cup for team highest in all products | Iowa State College |
| J. G. Cherry cup for team highest in butter | Iowa State College |
| Hoards Dairyman cup for team highest in cheese | Purdue University |
| J. B. Ford cup for team highest in milk | Massachusetts Agricultural College |
| National Federation of Milk Producers cup to institution having highest individual in milk | Iowa State College, W. W. Griese |

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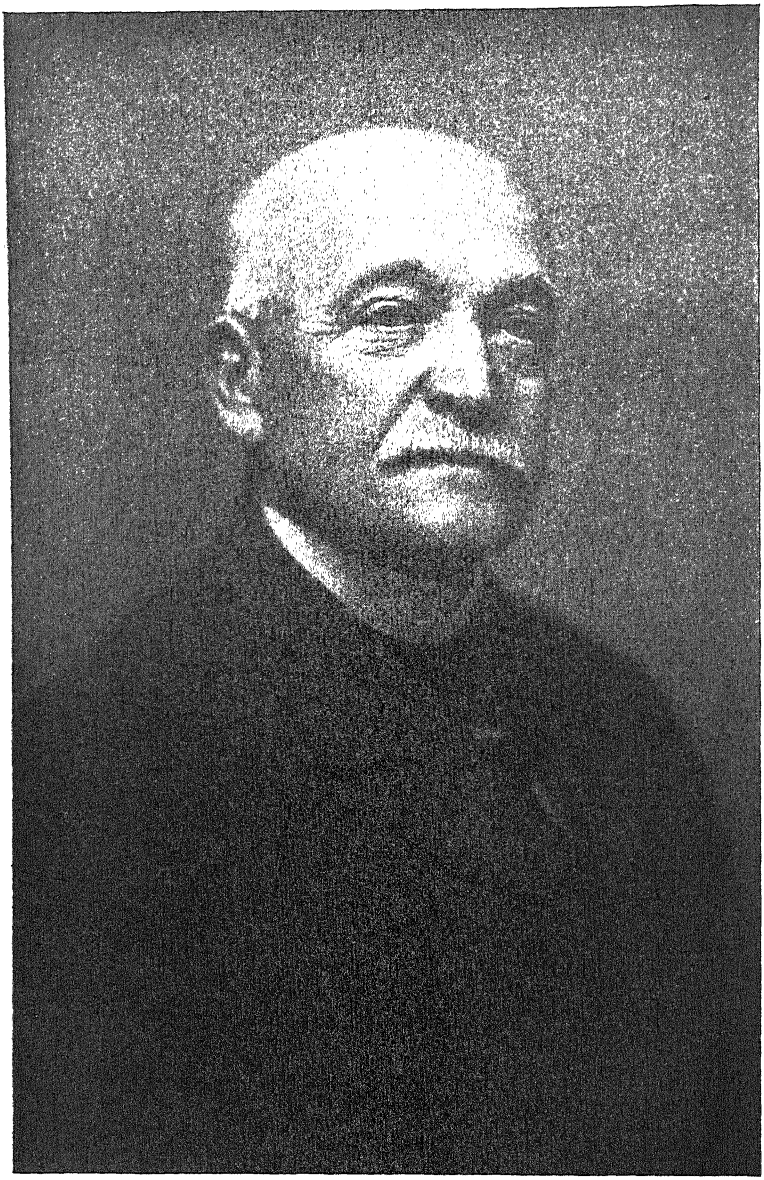
|---|---|
| Gold medal for highest individual all products, by National Dairy Exposition | Werner W. Griese, Iowa State College |
| Silver medal (second) | Roy O. Storvick, Iowa State College |
| Bronze medal (third) | Truman D. Romig, Ohio State University |
| Gold medal for highest individual in butter. American Creamery Butter Manufacturers Association | Roy O. Storvick, Iowa State College |
| Silver medal for second highest individual in butter N. D. E. | Fern T. Moore, Iowa State College |
| Bronze medal for third highest individual in butter, N. D. E. | Werner W. Griese, Iowa State College |
| Gold medal for highest individual in cheese National Cheese Association | F. H. Cherry, Purdue University |
| Silver medal for second highest individual in cheese, N. D. E. | Pierce Thompson, University of California |
| Bronze medal for third highest in cheese, N. D. E. | Chas. H. Gough, Pennsylvania State |
| Gold medal for highest individual in milk. International Milk Dealers Association | Werner W. Griese, Iowa State College |
| Silver medal for second highest in milk, N. D. E. | P. J. Davenport, Massachusetts Agricultural College |
| Bronze medal for third individual in milk, N. D. E. | Leland L. Currier, Massachusetts Agricultural College |



George A. Smith

# IN MEMORY OF GEORGE A. SMITH

Since we last met there has passed from among us one of the pioneers in American scientific dairying and the American Dairy Science Association expresses its appreciation of his life and work.

The Honorable George Alonzo Smith was born near Burlington, Otsego County, New York, on September 29, 1842. He intended to enter the ministry and received a thoro training in classical and religious literature.

While living on a farm to improve his health he became interested in the farm manufacture of cheese and because of his success with it, was soon making the milk of the neighborhood into cheese. In this way was developed one of the first cheese factories in the State of New York.

Upon the organization of a dairy division in the state government, his succses in the operation of a group of cheese factories led to his appointment as the first State Instructor in Cheese Making, probably the first appointment of its kind in the United States. For thirty-three years he remained a dairy teacher, a period of loyal service which has been rarely equaled.

His skill both as a teacher and as an administrator led to his appointment, in 1891, as Director of Farmers' Institutes and he helped lay the foundation of the present University Extension Work in Agriculture. In 1893 he directed the dairy exhibit for New York at the World's Fair at Chicago; in 1901 was in charge of the State dairy display at the Pan-American Exposition at Buffalo, and for years he took an active part in the dairy exhibit at the New York State Fair.

In connection with a reorganization of the New York State Agricultural Experiment Station there was created the position of Dairy Expert. Mr. Smith filled this position for twenty-three years, until his retirement in 1921. His work there was connected with studies of cheese ripening, tuberculosis and breeding problems of cattle, pasteurization and texture problems in butter, and various forms of teaching and inspection.

Quiet but vigorous, clean in mind and body, endowed with unusually good judgment, kindly almost to a fault, he was a man with a host of friends but singularly without enemies. To know him was to respect him.

# THE TRIER COMPARED WITH THE WEDGE METHOD OF SAMPLING TUB BUTTER*

H. B. ELLENBERGER

*University of Vermont, Burlington, Vermont*

AND

E. S. GUTHRIE

*Cornell University, Ithaca, New York*

This paper is a preliminary report of a study in which the wedge and trier methods of sampling butter were compared. Although this work has not been completed, a summary of the results thus far obtained is given here in the hope that it may be of some assistance to inspectors, manufacturers, and dealers who are interested in accurately determining the composition of butter.

One of the most difficult problems of almost any analysis is to obtain a representative sample. Many commonly accepted methods of sampling a particular product have not been subjected to a careful study as to their accuracy. This seems to have been the case with butter.

We do not know the exact history of the trier and wedge methods of sampling butter, but we do know that the butter dealers have not been satisfied with the wedge system of sampling because it defaced the package. Perhaps the inspectors also were inclined to prefer the trier method on account of the large amount of time required to upset the tubs and partially or wholly strip them of their liners, preparatory to taking the wedge samples. It seems evident, therefore, that the trier method is more acceptable to both the butter merchants and the inspectors. The Bureau of Internal Revenue has, in most cases, given the butter dealer his choice of procedure in sampling.

Since the Bureau of Chemistry has had charge of the enforcement of the fat law, some of its stations have directed that the

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* Received for publication January 20, 1925.

first samples should be taken with a trier. Then, if the package was suspected of being low in fat, it was to be sampled by the wedge method. From this it appears that the wedge system was generally considered more accurate; but a search of the literature has failed to reveal any convincing evidence of the truth of this opinion.

The old fat law of 82.5 per cent fat was antiquated many years ago; but on March 4, 1923, Congress passed a new law defining butter as follows:

> *Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,* That for the purposes of the Food and Drug Act of June 30, 1906 (Thirty-fourth Statutes at Large, page 768), "butter" shall be understood to mean the food product usually known as butter, and which is made exclusively from milk or cream, or both, with or without common salt, and with or without additional coloring matter, and containing not less than 80 per centum by weight of milk fat, all tolerances having been allowed for. Approved, March 4, 1923.

The administration of this act was placed in the hands of the Bureau of Chemistry in the Department of Agriculture, which had had charge of the enforcement of the old law. This Bureau immediately sought further information on proper sampling. Dr. H. W. Redfield, Chief of the New York Station, asked E. S. Guthrie to run a few determinations. At the same time, H. B. Ellenberger was coöperating with the Boston Station in a similar study of the two methods.

## THE TECHNIQUE OF SAMPLING AND TESTING

The technique of each investigator in taking the samples should be clearly understood.

The wedge samples taken by Ellenberger were as follows, according to instructions received by him from the Boston Station of the Bureau of Chemistry:

The butter was removed from the tub, and the mass was cut with a wire completely through at the junctions of the upper and middle thirds and of the lower and middle thirds. The bottom third was then removed, and a V-shaped piece was cut from each side of the middle section, extending from the circumference to the center of the tub. These two V-shaped pieces constituted the sample, which approximated one-half pound. This method was used by at least one station of the Bureau of Chemistry when there was good reason to believe that adulteration would be found.

All the samples taken by Guthrie were from butter that had been held at 42° to 55°F. He took the wedge samples with a knife, as instructed by the New York Station of the Bureau of Chemistry, making a four-sided plug, very much like a watermelon plug. These plugs were taken from opposite sides of a tub, at points not lower than one-fourth of the depth of the package from the bottom nor higher than the middle. The two portions together approximated one-half pound.

Ellenberger's trier sample consisted of two diagonal samples starting on opposite sides of the tub at the top and crossing near the center of the tub.

The vertical trier samples taken by Guthrie were as prescribed by the New York Station of the Bureau of Chemistry. A sample consisted of one trierful from the center of the tub, extending from the top to the bottom, and two other trierfuls, each taken half-way between the center plug and the edge of the package and diametrically opposite each other.

The diagonal trier sample taken by Guthrie was obtained by inserting the trier near the edge at the top and directing it to the bottom at the opposite side. This sample comprised only one trierful and contained about one-third as much butter as either his vertical trier or wedge samples.

The analyses made by Guthrie were determined by weighing the sample on a fine balance, sensitive to the fourth decimal place of a gram. Ten grams were weighed in an aluminum cup, and this sample was dried on an electric plate. The amount of fat in the residue, after the moisture had been thus evaporated, was determined by the Kohlman method.

Ellenberger also employed delicate balances, sensitive to the fourth decimal part of a gram. He followed the official method of analysis outlined by the Association of Official Agricultural Chemists. He used a water oven to drive off the moisture, until the samples ceased to lose weight. He made all his determinations in duplicate.

### THE RESULTS

Ellenberger first studied a lot of 34 tubs. In all but one test the wedge method showed more moisture than the trier. The maximum range of difference was from −0.15 to +1.35 per cent. That is, in one tub the wedge sample contained 0.15 per cent less moisture than the vertical trier sample, and in another tub the wedge sample showed 1.35 per cent more moisture than the vertical trier sample. The remaining samples ranged between these two extremes, the average variation being 0.69 per cent. The average percentage of moisture in the wedge samples was 0.68 per cent above that in the trier samples.

Guthrie found similar results in a series of 21 tubs, 6 of which were cold-storage goods. As in Ellenberger's analyses, in only one tub did the wedge sample show less moisture than the vertical trier sample. The maximum range of difference was from −0.19 to +0.82 per cent. The remaining samples ranged between these two extremes, the average variation being 0.38 per cent. The average percentage of moisture in the wedge samples was 0.36 per cent above the vertical trier samples.

Thus, what seemed to be a simple analytical problem became a complicated one. The question immediately arose, Which method of sampling is the more nearly correct? In the meantime, the Bureau of Chemistry had directed its inspectors to employ the trier system. Thus the buttermaker and his associates in the butter industry were given the benefit of the doubt.

Both investigators decided that the most nearly accurate sample would be obtained by putting the entire tub of butter into a dry, ten-gallon milk can and working it up in the same ways as the smaller trier and wedge samples had been worked. The whole tub of butter was softened by holding it in water at 90°F. for twelve to fourteen hours in a tightly closed can. It was then very thoroughly stirred to a creamy consistency.

Ellenberger used a can with a hole in the top through which the agitator could be operated, so that very little moisture escaped while the sample was being stirred. Guthrie stirred his sample with a long-handled paddle in a fairly humid atmosphere.

In this phase of the investigation, Ellenberger worked with 16 tubs of butter from several different creameries. He took two sets of trier samples and two sets of wedge samples from each tub. He then prepared the whole tub as described above, and took two samples and analyzed them separately. Altogether, he sampled each tub six times, and analyzed each sample in duplicate. Ellenberger found, in 31 comparisons from these 16 tubs, that the wedge sample showed an average of 0.07 per cent less moisture than the entire-tub sample, and that the trier sample (in this series all free moisture on the back of the trier was included) contained 0.17 per cent less moisture than did the entire-tub sample. The maximum range of difference was from −0.91 to +0.42 per cent for the trier method as compared with the entire-tub sample, and from −0.53 to +0.77 per cent for the wedge method as compared with the entire-tub sample. The average variation from the entire-tub analysis was 0.31 per cent for the vertical trier sample and 0.23 per cent for the wedge sample. However, 6 of these tubs, comprising twelve sets of samples, were unsalted butter. Separating the results of the salted and unsalted butter, we have the following:

From 10 tubs of salted butter 19 sets of samples were taken. The average analysis of the vertical trier samples was 0.34 per cent less, and of the wedge samples 0.11 per cent less, than the entire-tub samples. The maximum range of difference was from −0.91 to +0.21 per cent for the vertical trier samples, with an average variation of 0.39 per cent; and from −0.53 to +0.77 per cent for the wedge samples, with an average variation of 0.25 per cent. The remaining 6 tubs of unsalted butter gave 12 sets of samples. The average analysis of the vertical trier samples was 0.09 per cent less, and of the wedge samples .01 per cent less, than the whole-tub samples. The maximum range of difference was from −0.38 to +0.42 per cent for the trier

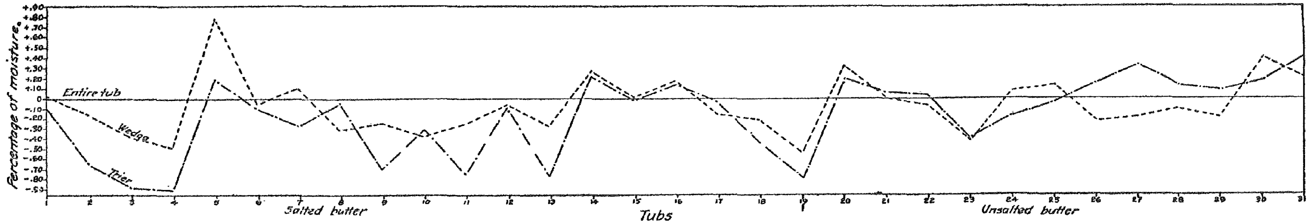

FIG. 1. VARIATIONS OF MOISTURE BY ELLENBERGER

The straight dark line is the entire-tub sample which is assumed to be correct. The other lines show the variations in moisture content from this assumed correct analysis.

The maximum range of difference was as follows: Vertical trier sample, −0.91 to +0.42 per cent; wedge sample, −0.53 to +0.77 per cent.

The average variations of both plus and minus analyses were: Wedge sample, 0.23 per cent; vertical trier sample, 0.31 per cent.

Finally and most important: The wedge sample contained an average of 0.07 per cent, and the vertical trier sample 0.17 per cent, less moisture than the entire-tub sample.

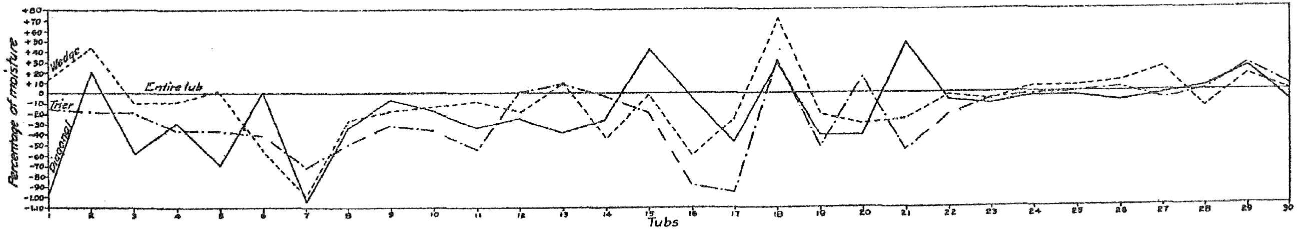

FIG. 2. VARIATIONS OF MOISTURE BY GUTHRIE

The straight dark line is the entire-tub sample which is assumed to be correct. The other lines show the variations in moisture content from this assumed correct analysis.

The maximum range of difference was as follows: Diagonal trier sample, −1.05 to +0.47 per cent; wedge sample, −1.02 to +0.47 per cent; vertical trier, −0.95 to +0.32 per cent.

The average variations of both plus and minus analyses were: Wedge sample, 0.24 per cent; vertical trier sample, 0.29 per cent; diagonal trier sample, 0.30 per cent.

Finally and most important: The wedge sample contained an average of 0.10 per cent, the diagonal trier sample 0.18 per cent, and the vertical trier sample 0.22 per cent less moisture than the entire-tub sample.

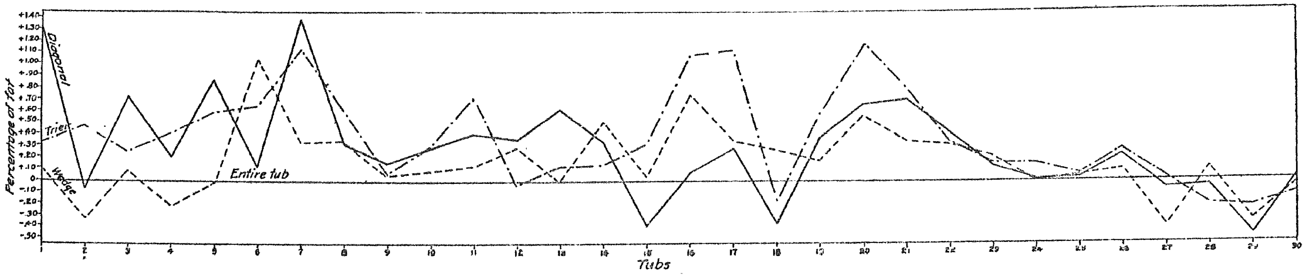

FIG. 3. VARIATIONS OF MILK FAT BY GUTHRIE

The straight dark line is the entire-tub sample which is assumed to be correct. The other lines show variations in milkfat content from this assumed correct analysis.

The maximum range of difference was as follows: Diagonal trier sample, −0.40 to +1.31 per cent; wedge sample −0.39 to +1.03 per cent; vertical trier sample, −0.22 to +1.17 per cent.

The average variations of both plus and minus analyses were: Diagonal trier sample, 0.39 per cent; wedge sample, 0.25 per cent; and vertical trier sample, 0.42 per cent.

Finally and most important: The wedge sample contained an average of 0.16 per cent, the diagonal trier 0.29 per cent, and the vertical trier 0.36 per cent, more fat than the entire-tub sample.

samples with an average variation of 0.19 per cent; and from −0.40 to +0.41 per cent for the wedge samples, with an average variation of 0.19 per cent.

As already noted, Guthrie, using a series of 30 tubs, compared diagonal trier, wedge, and vertical trier samples with an entire-tub sample. He studied the variation of both moisture and fat. He found, in the moisture analyses, that the wedge sample compared more favorably with the entire-tub than did either of the others. The average of the 30 tubs showed the wedge sample to contain 0.10 per cent less moisture, the diagonal trier sample 0.18 per cent less moisture, and the vertical trier sample 0.22 per cent less moisture, than the entire-tub sample. The maximum range of difference was from −1.05 to +0.47 per cent for the diagonal, from −1.02 to +0.72 per cent for the wedge, and from −0.95 to +0.32 per cent for the trier, with average variations of 0.30, 0.24, and 0.29 per cent, respectively. The fat analyses also showed the wedge sample to be the closest to the entire-tub sample. The wedge contained 0.16 per cent, the diagonal 0.29 per cent, and the trier 0.36 per cent more moisture than the entire-tub sample.

In order that these data may be visualized, figures 1, 2, and 3 have been prepared. Figure 1 shows the variations in moisture, from package to package, in the 31 sets of samples from the 16 tubs sampled and analyzed by Ellenberger. Figures 2 and 3 show the variations in the moisture and fat in the 30 tubs studied by Guthrie.

In regard to the accuracy of the analyses, most of the time the difference between the first analysis and its duplicate was less than 0.05 per cent.

## SUMMARY

The wedge method of sampling seems to be the most nearly accurate, as shown by Ellenberger's moisture analyses and Guthrie's moisture and fat analyses; but it also seems reasonable to take a diagonal sample first, because this is easily obtained without removing much butter from the package. If this sample gives indications of a low fat content, a second sample can then be taken by the wedge method.

## PHASES NOT YET REPORTED

As has already been stated, further research on this problem is necessary. Ellenberger has found that there is a distinct difference in the moisture content when different triers are used. Perhaps this is also true when different knives are used to take the wedge samples. Guthrie has been paying special attention to the working of the butter. These, and other related problems, are still being studied.

# YEAST AS A SUPPLEMENTARY FEED FOR LACTATING COWS[1]

C. H. ECKLES AND V. M. WILLIAMS

*Division of Dairy Husbandry, University of Minnesota, St. Paul, Minnesota*

In a previous report results were given of the addition of yeast to the rations of growing calves. A second part of the investigation concerned the effect of using yeast as a supplementary feed for cows in milk and the influence upon the vitamin B content of the milk produced.

The requirements of the bovine for vitamins have not been determined as yet. Until sufficient experimental evidence is available to justify a different conclusion it would appear advisable to assume that this species has a vitamin requirement in general similar to that of laboratory animals, which have supplied the data upon which the present knowledge of vitamins is based.

The rations commonly fed cows in well managed dairy herds apparently would supply a liberal amount of vitamin B. At least half the nutrients are usually supplied in roughages which undoubtedly contain a fair amount of vitamin B. The remainder of the ration usually consists of ground grains and by-products. Seeds are looked upon as supplying a liberal amount of vitamin B, and when ground corn, oats, barley, or other grains are used it is certain the ration will contain vitamin B in considerable amounts. It would appear that the only possibility of a ration low in vitamin B would occur when by-products low in this accessory were used in excess. Gluten meal and beet pulp are examples of by-products low in this vitamin.

The logical procedure in studying the relation of a vitamin to the feeding of dairy cattle would appear to be as follows.

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[1] Received for publication July 9, 1924.

*a.* Determine if the bovine requires the vitamin in question as do the laboratory animals.

*b.* An estimation of the quantitative requirements in terms of some feeding stuff serving as an especially potent source of the vitamin.

*c.* Determine if any danger exists of ordinary rations being deficient in the vitamins in question.

*d.* Find if any advantage results from supplying the vitamin in amount considerably in excess of the minimum demands of the animals and of the amount usually received through the ration.

The study which is reported in this paper for reasons which were explained in the first report did not follow the procedure suggested but began with the last rather than the first of the four parts outlined. Large quantities of spent yeast are available from breweries and the use of this product as a feed for animals has long been common in Europe. In Europe yeast is looked upon as a source of efficient protein rather than a supplementary feed having special effects such as might be expected if it were fed for the sake of a vitamin it might furnish.

According to Sherman and Smith "While often referred to as growth promoting it should be emphasized that vitamin B is essential to normal nutrition at all ages."

"The mechanism of the action of vitamin B has been variously thought to be that of a physiological stimulant to glandular secretion of an indirect metabolic stimulant, particularly for carbohydrates." "While the relation of vitamin B to cellular metabolism seems still a matter of speculation, it is clear that it often exerts a very direct influence upon appetite."

## EXPERIMENTAL

With these facts in mind it appeared worth while to test the value of yeast as a supplement to a common ration as fed under practical conditions. The results were to be measured from the practical feeds standpoint as indicated specifically by effect upon milk production and in general upon condition and appetite of the animal as noted by observation. If the yeast possesses properties of a galactogog its addition to the ration should result in a stimulation of the mammary glands or by increasing the efficiency of the digestive and assimilative functions. The former might cause an increased appetite to compensate for the increased secretion while the latter might make an increased secretion possible by furnishing the mammary glands with more nourishment.

The plan of the experiment was to feed commercial dry yeast at the rate of 25 grams daily for each pound of milk produced. This particular amount was chosen arbitrarily but as at least furnishing even more than the maximum amount that could be fed under commercial conditions. Eight cows were used, arranged in two groups and fed according to the reversal plan as follows:

| | GROUP 1 | GROUP 2 |
|---|---|---|
| Period 1 (40 days).................... | Yeast supplement | Basal ration |
| Period 2 (40 days).................... | Basal ration | Yeast supplement |
| Period 3 (40 days).................... | Yeast supplement | Basal ration |

The first ten days of each period were considered preliminary. The comparison of data in each case is for the thirty-day periods following the preliminary. The ration used consisted of alfalfa hay, corn silage, dried beet pulp and a grain mixture of ground corn 2 parts, ground oats 2 parts, wheat bran 2 parts, and linseed oilmeal 1 part. The plan of feeding in general was to give each animal as much roughage as she would consume readily and in addition a supply of grain somewhat more than necessary to supply the protein and energy needed, according to Armsby's standards. The purpose in supplying some surplus of nutrients was to allow any stimulation from the yeast to exert its full effect without the limitation from a deficiency of nutrients. The energy fed ranged from 120 to 135 per cent of that called for by the feeding standard. A summary of milk and fat produced by the individuals used is given in table 1. The reversal plan of arranging experimental periods of feeding recognizes the fact that after the first month under normal conditions the decline in milk production follows a rather uniform curve until the end of the lactation period approaches. The average of the first and the third period should, therefore, be practically the same as the figure for the second period. The object in arranging one group with the experimental ration the first and the third, while for the other group the experimental ration in the second period is to check environmental factors such as weather conditions, that might otherwise not be detected. The results in table 1 do not indicate any effect from the addition of yeast to the ration. In the first group the average milk yield for the yeast periods was 26.4 pounds per day and for the second without yeast 26.5. In the second group the average of the two

TABLE 1

*Summary of milk and fat production*

| ANIMAL NUMBER | MILK PER DAY | | | BUTTERFAT PER DAY | | |
|---|---|---|---|---|---|---|
| | First 30-day period | Second 30-day period | Third 30-day period | First 30-day period | Second 30-day period | Third 30-day period |
| | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* |
| *Group 1* | | | | | | |
| 120 | 24.46* | 22.47 | 19.95* | 1.181* | 1.115 | 1.035* |
| 301 | 39.84* | 37.80 | 37.85* | 1.135* | 1.026 | 1.097* |
| 317 | 23.06* | 20.64 | 11.75* | 0.872* | 0.752 | 0.503* |
| 320 | 27.41* | 27.13 | 26.78* | 0.922* | 0.877 | 0.918* |
| Average | 28.69* | 26.51 | 24.08* | 1.027* | 0.942 | 0.888* |
| Average for first and third periods | | 26.38 | | | 0.957 | |
| *Group 2* | | | | | | |
| 102 | 10.99 | 8.46* | 8.73 | 0.509 | 0.428* | 0.456 |
| 306 | 33.89 | 32.17* | 31.91 | 1.190 | 1.024* | 0.988 |
| 333 | 34.20 | 31.32* | 26.05 | 1.077 | 1.024* | 0.875 |
| 500 | 32.57 | 29.13* | 26.57 | 1.232 | 1.115* | 1.078 |
| Average | 27.91 | 25.27* | 23.31 | 1.002 | 0.897* | 0.849 |
| Average for first and third periods | | 25.61 | | | 0.925 | |

* Receiving 25 grams dried yeast per pound of milk.

basal periods was 25.6 pounds compared to 25.5 for the yeast period.

## CONCLUSIONS

Adding a dried yeast preparation at the rate of 25 grams per pound of milk produced to a ration commonly fed in good dairy herds did not increase the milk or butterfat yield.

No effect could be observed in the way of condition of the animal or stimulation of appetite.

# THE EFFECT OF CANE MOLASSES ON THE DIGESTIBILITY OF A COMPLETE RATION FED TO DAIRY COWS*

PAUL S. WILLIAMS

*Department of Dairy Husbandry, Pennsylvania State College, State College, Pennsylvania*

Cane molasses has been used as feed for live stock for many years. It has served especially as an appetizer or for increasing the palatability of grains and roughages. A few experiments have been run by various stations to determine the feeding value of molasses as compared with grains, while a larger number have been run to determine its effect on the digestibility of the ration.

The United States produced in 1919 over sixteen million gallons of cane molasses, and imported approximately 160 million gallons. (1) Part of this is used for the manufacture of alcohol, small quantities for domestic consumption and the remainder, which as nearly as the writer can determine amounts to approximately 10 to 15 per cent, goes for stock feeding purposes.

## SUMMARY OF PREVIOUS EXPERIMENTS

The results reported by Lindsey and Smith (2) indicate that cane molasses in the ration as a general rule depresses digestibility. Patterson and Outwater (3) got results which conflict with those of Lindsey and Smith. Their investigations indicated a beneficial effect on digestibility. As a result of the favorable report made by Patterson and Outwater, extravagant claims have been put forth by companies dealing in molasses as to its value in increasing the digestibility of the other feeds in the ration. In view of the fact that sufficient experimental data to settle the question definitely have not been established, and that some of the results are conflicting, the following investigation was planned in order to give further light on the question.

* Received for publication January 8, 1925.

## OBJECT OF EXPERIMENT

The object of this investigation was to determine the effect of cane molasses on the digestibility of a complete ration fed to dairy cows.

TABLE 1

| COW NUMBER | AGE JANUARY 18, 1923 | DATE OF LAST FRESHENING | WEIGHT AT BEGINNING OF EXPERIMENT* | DATE BRED | MILK PER DAY AT BEGINNING OF EXPERIMENT* |
|---|---|---|---|---|---|
| 848 | 7 years, 299 days | 11/21/22 | 1198 | 4/ 4/23 | 50 |
| 860 | 6 years, 213 days | 12/10/22 | 1256 | 4/25/23 | 37 |
| 855 | 7 years, 50 days | 11/30/22 | 1556 | 4/ 2/23 | 59 |
| 1117 | 5 years, 33 days | 12/24/22 | 1153 | 3/19/23 | 47 |

* Average of first three days of the experiment.

TABLE 2

| | AMOUNT |
|---|---|
| | *pounds* |
| Corn meal | 200 |
| Wheat bran | 150 |
| Ground oats | 150 |
| Linseed meal (old process) | 75 |
| Peanut meal | 100 |
| Diamond gluten meal | 50 |
| Salt | 5 |

## PLAN AND PROCEDURE OF EXPERIMENT

The general plan of the experiment was as follows:

*Series A—Trial 1:* Check trial. Four pure bred Holstein cows were fed a grain ration with hay and silage.

*Series A—Trial 2:* The same four cows were fed as in trial one with the addition of sufficient molasses to make up 15 per cent of the total concentrates.

*Series A—Trial 3:* The same cows were again fed as in trial one with the addition of sufficient molasses to make up 25 per cent of the total concentrates.

Trials one, two and three were repeated and designated as series B. The three trials were again repeated and designated as series C. A total of nine trials were thus included in series A, B and C.

The amounts of molasses fed in trial number two of each series is, according to general feeding practice, a medium amount. The amounts fed in trial three of each series is a large amount.

Each trial covered a period of twenty-one days which was divided into a preliminary feeding period of ten days and a collection period of eleven days.

The experiment was begun January 18, 1923 with series A—trial 1, and ended at the close of series C—trial 3, July 25, 1923.

It will be observed that a complete ration for dairy cows in milk was fed throughout the whole experiment. At the beginning of the experiment a special effort was made to balance up the rations according to the maintenance and milk production requirements of the Armsby feeding standard.

### COWS USED IN THE EXPERIMENT

Table 1 shows how the cows compared with each other.

The grain ration fed during the entire experiment was made up as shown in table 2.

Using the average feed analysis from Pennsylvania Bulletin 161, 1 pound of this mixture contains 0.169 pounds true digestible protein and 0.759 therms net energy.

A quantity of the grain mixture sufficient to last through six trials was prepared before the experiment started. This grain was thoroughly mixed and then stored in bags ready for use. Just previous to each of the trials, the individual feeds were weighed up into lard pails with tight fitting lids. A total of two hundred fifty-two feeds were so prepared at the beginning of each twenty-one day trial. A composite sample for chemical analysis was taken at the time individual feeds were weighed.

Alfalfa of rather low quality was fed to the cows in series A and B. In series C, mixed hay of good quality, approximately 60 per cent clover and 40 per cent timothy, was fed. The hay was prepared for feeding and thorough mixing by cutting it into short lengths. Two hundred fifty-two individual feeds of hay were weighed into bags just previous to each trial. A composite sample for chemical analysis was taken at this time.

The silage was of good quality. It was weighed fresh from the silo each day in individual feeds. Samples of equal size were taken each day and put immediately into the drying oven.

All feed samples were dried as completely as possible in the steam heated oven. They were then removed and allowed to stand for a few days until their moisture content came into equilibrium with the atmospheric moisture. Finally they were weighed and ground in preparation for chemical analysis.

One analysis of cane molasses was made from a composite sample taken dairly during the entire experiment.

No appreciable gain or loss in weight of the cows occurred throughout the nine trials of the experiment. This fact is an indication that the ration supplied about the correct amount of energy.

Using the analyses given in Pennsylvania Bulletin 161, the rations fed to each cow satisfied the Armsby Feeding Standard both for protein and energy at all times.

The cows were all in fair to good conditions at the beginning of the experiment and remained so throughout the nine trials.

When a cow refused any part of her daily feed she was immediately removed from the experiment until such time as she consumed the regular ration, and then she was not included in any digestion trial unless she had received the ration without change in amount for at least ten days previous to the beginning of the trial. Two of the cows No. 855 and No. 1117 went through the entire experiment without missing a feed. Cow No. 860 was off feed and therefore not included in trial 2, series B, and trial 3, series C. Cow No. 848 was off feed during trial 2, series C.

## COLLECTION OF EXCRETA AND METHOD OF TAKING SAMPLES

Two men were in attendance at all times during the collection period. All dung voided by each cow was caught in a scoop shovel, a separate shovel being used for each cow. The dung collected was weighed once each day at 8'oclock a.m. Care was taken that exactly ten days were included in each trial.

The urine was swept down the drain as voided. Since straw or shavings could not be used as bedding, burlap bags were tacked to the cork brick floor on which the cows stood. These were changed at frequent intervals, so that the cows had a fairly comfortable bed at all times.

In order that the digestion trials be run under as nearly normal conditions as possible the first day of the eleven days trial was discarded. This preliminary day was allowed so that the cows might recover from any excitement likely to be caused at the beginning of a digestion trial.

Exact aliquot samples of dung were taken daily and preserved as a composite for the ten-day period. At the close of this period a portion of the sample was used for nitrogen determinaminations by the Köenig method. The remaining part of the sample was oven dried and ground for determination of the other constituents. The method of drying and grinding conformed to that described above concerning feed samples.

## CARE OF THE COWS

The stalls used were provided with steel mangers with high sides to prevent the cows from eating the feed that belonged to adjacent animals. Feeding and watering were done at regular intervals three times daily immediately before milking. In addition to the regular watering the cows were given an opportunity to drink each day immediately after weighing at 10 o'clock a.m. The hay, silage, grain, and molasses were all fed together at one time, the hay being placed in the bottom of the manger and the other feeds on top of it in the order mentioned. The molasses was dissolved in about double its weight of warm water and was poured over the other feeds after they were put into the manger.

## MILK PRODUCTION

A study of the milk production shows that the cows gradually fell off during the entire experiment at a rate about equal to what one would naturally expect under conditions of advance in lactation.

TABLE 3

*Digestibility of nutrients by trials*

| SERIES | TRIAL | DATE | MOLASSES IN DRY MATTER OF RATION | CRUDE PROTEIN | CRUDE FIBER | NITROGEN FREE EXTRACT | ETHER EXTRACT | DRY MATTER |
|---|---|---|---|---|---|---|---|---|
| | | | | **Cow 848** | | | | |
| | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| A 1 | Check | 1/29–2/ 7 | | 69.678 | 44.608 | 71.199 | 77.110 | 64.576 |
| A 2 | Molasses | 2/19–2/28 | 5.202 | 63.648 | 42.717 | 70.188 | 72.340 | 62.624 |
| A 3 | Molasses | 3/12–3/21 | 9.856 | 61.218 | 54.166 | 67.033 | 65.670 | 61.702 |
| B 1 | Check | 4/ 1–4/10 | | 65.436 | 55.904 | 70.695 | 70.895 | 65.328 |
| B 2 | Molasses | 4/23–5/ 2 | 4.918 | 63.410 | 46.900 | 71.410 | 75.123 | 63.690 |
| B 3 | Molasses | 5/14–5/23 | 9.684 | 60.646 | 53.641 | 69.306 | 74.877 | 62.933 |
| C 1 | Check | 6/ 4–6/13 | | 63.047 | 56.903 | 71.906 | 71.727 | 66.150 |
| C 2 | * | 6/25–7/ 4 | | | | | | |
| C 3 | Molasses | 7/16–7/25 | 12.386 | 61.460 | 65.330 | 71.650 | 72.448 | 64.053 |
| | | | | **Cow 860** | | | | |
| A 1 | Check | 1/29–2/ 7 | | 66.439 | 45.366 | 71.640 | 56.876 | 65.011 |
| A 2 | Molasses | 2/19–2/28 | 5.202 | 62.616 | 42.717 | 70.719 | 72.340 | 62.545 |
| A 3 | Molasses | 3/12–3/21 | 9.856 | 59.684 | 44.979 | 70.894 | 81.854 | 62.024 |
| B 1 | Check | 4/ 1–4/10 | | 67.291 | 54.256 | 71.642 | 70.722 | 65.906 |
| B 2 | * | 4/23–5/ 2 | | | | | | |
| B 3 | Molasses | 5/14–5/23 | 9.684 | 57.641 | 50.878 | 70.242 | 71.008 | 62.404 |
| C 1 | Check | 6/ 4–6/13 | | 63.047 | 56.903 | 71.906 | 71.727 | 66.150 |
| C 2 | Molasses | 6/25–7/ 4 | 5.247 | 56.666 | 54.032 | 67.912 | 65.368 | 61.332 |
| C 3 | * | 7/16–7/25 | | | | | | |
| | | | | **Cow 855** | | | | |
| A 1 | Check | 1/29–2/ 7 | | 69.601 | 50.003 | 76.609 | 78.255 | 67.197 |
| A 2 | Molasses | 2/19–2/28 | 5.893 | 63.398 | 41.050 | 70.980 | 73.617 | 62.938 |
| A 3 | Molasses | 3/12–3/21 | 10.569 | 58.114 | 47.455 | 70.030 | 58.138 | 61.019 |
| B 1 | Check | 4/ 1–4/10 | | 65.410 | 48.899 | 68.900 | 75.706 | 62.727 |
| B 2 | Molasses | 4/23–5/ 2 | 5.914 | 62.158 | 49.077 | 72.660 | 66.610 | 62.362 |
| B 3 | Molasses | 5/14–5/23 | 10.356 | 58.387 | 55.162 | 70.686 | 73.850 | 63.039 |
| C 1 | Check | 6/ 4–6/13 | | 60.799 | 54.382 | 71.526 | 72.481 | 64.899 |
| C 2 | Molasses | 6/25–7/ 4 | 5.035 | 61.685 | 58.623 | 71.851 | 73.889 | 65.740 |
| C 3 | Molasses | 7/16–7/25 | 11.215 | 59.371 | 60.684 | 71.732 | 69.114 | 59.972 |

* Off feed.

TABLE 3—*Continued*

| SERIES | TRIAL | DATE | MOLASSES IN DRY MATTER OF RATION | CRUDE PROTEIN | CRUDE FIBER | NITROGEN FREE EXTRACT | ETHER EXTRACT | DRY MATTER |
|---|---|---|---|---|---|---|---|---|
| Cow 1117 | | | | | | | | |
| | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| A 1 | Check | 1/29–2/ 7 | | 62.554 | 44.492 | 71.692 | 70.646 | 63.330 |
| 2 | Molasses | 2/19–2/28 | 5.202 | 59.661 | 39.519 | 68.858 | 73.385 | 71.323 |
| 3 | Molasses | 3/12–3/21 | 9.856 | 58.427 | 51.613 | 68.693 | 72.965 | 64.550 |
| B 1 | Check | 4/ 1–4/10 | | 64.434 | 53.919 | 74.197 | 71.953 | 67.200 |
| 2 | Molasses | 4/23–5/ 2 | 4.918 | 59.592 | 51.439 | 73.093 | 68.593 | 63.690 |
| 3 | Molasses | 5/14–5/23 | 9.684 | 56.980 | 52.028 | 71.643 | 74.442 | 63.371 |
| C 1 | Check | 6/ 4–6/13 | | 60.799 | 54.382 | 71.526 | 72.481 | 64.899 |
| 2 | Molasses | 6/25–7/ 4 | 5.247 | 58.306 | 58.479 | 69.884 | 64.980 | 63.648 |
| 3 | Molasses | 7/16–7/25 | 9.882 | 63.388 | 61.917 | 77.217 | 76.530 | 71.729 |

## RESULTS OF DIGESTION TRIALS

The summary of results of the nine different digestion trials were computed in the usual way from the data. It should be explained here that it was assumed that molasses was completely digested. (2) Hence, the nutrients in the molasses were not added in as a part of the food consumed. This assumption permits of a direct comparison of the nutrients intake with that excreted in the dung, in determining the percentage of digestibility.

One encounters considerable difficulty in trying to interpret the results shown by data in table 3. It is observed that no one fact seems to hold true consistently throughout all the trials either for individual cows or for all cows.

Taking crude protein as an example, the molasses trials for the cows 848 and 860 show a depression in the digestibility, but on the other hand the cows 855 and 1117 show slight increases in digestibility.

The figures for digestibility of crude fiber show no regularity of increase or decrease of digestibility in relation to the amount of molasses consumed. The same can be said of ether extract and nitrogen free extract. A decrease in digestibility where molasses was fed however appears to be evident when one examines the dry matter data. Taking cow 848 Group A for example, the check trial showed about 64.6 per cent; with 5.2 per cent of molasses in the dry matter, the corresponding figure is 62.6 per cent; and with 9.8 per cent molasses in the dry matter, the percentage digestibility is 62 per cent. Similar results are shown in series B and C for this cow. Studying the data of the other three cows, we find exactly the same thing with but three exceptions, viz., cow 855 in series B and cow 1117 in series A and C. In the first mentioned exception, it is seen that the low percentage of molasses causes the usual decrease, but the high percentage of molasses shows a digestibility even higher than the check trial. Exactly the same comment applies to the third mentioned exception. In the second exception, the low percentage molasses ration has a higher percentage of digestibility than the check trial while the high percentage molasses ration has also a slightly higher percentage of digestibility than the check trial. To sum up the dry matter data as shown in table 3, it is noted that out of the twenty-one possible comparisons of the different molasses trials with their respective check trials, 76.2 per cent of the molasses trials show a depressed digestibility. In the eight cases where de-

TABLE 4

*Average digestibility for all cows by trials*

| SERIES | | TRIALS | CRUDE PROTEIN | CRUDE FIBER | ETHER EXTRACT | NITROGEN FREE EXTRACT | DRY MATTER |
|---|---|---|---|---|---|---|---|
| | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| A | 1 | Check | 67.482 | 46.205 | 71.205 | 72.074 | 65.101 |
| | 2 | Molasses | 62.369 | 41.191 | 73.013 | 72.227 | 64.798 |
| | 3 | Molasses | 59.338 | 49.362 | 69.278 | 69.190 | 62.284 |
| B | 1 | Check | 65.634 | 53.147 | 72.437 | 71.279 | 65.211 |
| | 2 | Molasses | 52.469 | 58.366 | 69.942 | 72.398 | 63.230 |
| | 3 | Molasses | 58.412 | 52.443 | 73.555 | 70.476 | 62.940 |
| C | 1 | Check | 62.243 | 51.425 | 68.549 | 69.116 | 62.767 |
| | 2 | Molasses | 48.897 | 57.076 | 68.139 | 69.897 | 54.639 |
| | 3 | Molasses | 61.385 | 62.587 | 72.670 | 73.695 | 65.616 |

pressions are noted with both high and low percentages of molasses, it is seen that in six instances the greatest depression was caused by the greater amount of molasses, while in two instances the greater depression was caused by the smaller amount of molasses.

In order to study the data from the standpoint of averages, tables 4, 5 and 6 have been prepared. Table 4 presents an average percentage digestibility of the constituents.

The crude protein figures appear to indicate that molasses depressed digestibility since the digestibility of the protein in the check trial of each series ran higher than the corresponding molasses trials. With the exception of one average trial on high molasses in series C, the same comment may be offered concerning the dry matter data. The crude fiber figures show that the digestibility was increased by the molasses in four cases while in two cases it was decreased. The digestibility of the ether extract on the other hand is decreased by the molasses as often as it is increased.

Table 5 presents average figures for each of the four cows used in the experiment.

The method used in obtaining these figures may be illustrated as follows:

The respective amounts of feed consumed and digested by each animal in trial one of the three series of trials were added together and the average percentage digestibility was then worked by the usual method of computation. The average figures for trials two and three were obtained in the same way.

Before making a study of table 5 it should be pointed out that cow 848 was off feed in trial two, series C and cow 860 was off feed in trial three, series C. No data was obtained from these two cows in the trials mentioned, and consequently the figures Nos. 848 and 860 represent the average of two trials instead of three.

A study of the data presented here indicates that the molasses had no uniform effect on the digestibility of the different constituents. In six out of eight comparisons in the protein figures the percentage of digestibility is reduced by the molasses. In

TABLE 5

*Average digestibility for cows by combining like trials in each series*

| A-B-C | TRIALS | AVERAGE PERCENTAGE OF MOLASSES IN DRY MATTER OF RATION | CRUDE PROTEIN | CRUDE FIBER | ETHER EXTRACT | NITROGEN FREE EXTRACT | DRY MATTER |
|---|---|---|---|---|---|---|---|
| Cow 848* | | | | | | | |
| | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 1-1-1 | Check | | 66.307 | 52.448 | 73.457 | 70.457 | 65.329 |
| 2-2 | Molasses | 5.202 | 66.531 | 45.002 | 73.724 | 70.783 | 63.173 |
| 3-3-3 | Molasses | 10.509 | 61.117 | 57.433 | 71.423 | 69.190 | 62.802 |
| Cow 860† | | | | | | | |
| 1-1-1 | Check | | 65.152 | 52.185 | 66.227 | 71.731 | 65.686 |
| 2- -2 | Molasses | 5.138 | 59.818 | 48.297 | 68.706 | 69.346 | 61.941 |
| 3-3- | Molasses | 9.769 | 61.117 | 57.433 | 71.423 | 69.190 | 62.802 |
| Cow 855 | | | | | | | |
| 1-1-1 | Check | | 66.120 | 51.698 | 74.597 | 71.050 | 64.921 |
| 2-2-2 | Molasses | 5.017 | 62.474 | 49.963 | 71.431 | 75.097 | 65.773 |
| 3-3-3 | Molasses | 10.670 | 58.549 | 53.650 | 67.813 | 70.788 | 61.449 |
| Cow 1117 | | | | | | | |
| 1-1-1 | Check | | 62.649 | 51.003 | 71.687 | 72.461 | 65.160 |
| 2-2-2 | Molasses | 5.118 | 59.217 | 50.389 | 66.503 | 73.439 | 67.975 |
| 3-3-3 | Molasses | 9.806 | 64.667 | 54.728 | 74.814 | 72.598 | 66.524 |

* Cow 848 off feed in Series C—Trial II.

† Cow 860 off feed in Series B—Trial II and Series C—Trial III.

TABLE 6

*Average digestibility of the four cows in the three average trials*

| SERIES | TRIALS | CRUDE PROTEIN | CRUDE FIBER | ETHER EXTRACT | NITROGEN FREE EXTRACT | DRY MATTER |
|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 1 | Check | 65.524 | 50.223 | 70.857 | 70.978 | 64.622 |
| 2 | Molasses | 61.461 | 51.816 | 60.529 | 71.624 | 61.358 |
| 3 | Molasses | 59.480 | 47.029 | 71.991 | 70.838 | 63.337 |

the other two trials it is increased. In the case of crude fiber the greater amount of molasses seems to raise the percentage of digestibility while the smaller amount decreases it. This holds true for each cow.

The variations in the digestibility of ether extract and nitrogen free extract are very irregular and hence these data furnish no evidence as a basis for conclusions.

The digestibility of the dry matter is reduced by the molasses in both trials of cows 848 and 860, whereas it is increased in both trials with cow 1117. Cow 855 shows an increase in trial two and a decrease in trial three.

Still another angle for studying the data is presented in table 6. These average figures were obtained by the method used in computing those of table 5. Table 6 goes just a step further and presents the average of the four cows for each constituent. It is in reality a general summary of all the data.

It is observed that the digestibility of crude protein and dry matter are reduced by both medium and high amounts of molasses. The crude fiber and nitrogen free extract are increased by the medium amount, and decreased by the large amount of molasses, while the reverse of this statement applies to ether extract.

## CONCLUSIONS

The data of this experiment indicates that the digestibility of the crude fiber, the nitrogen free extract, and the ether extract is not uniformly affected in either direction by the presence of molasses in the ration. Molasses tends to depress the digestibility of crude protein and dry matter. However, this depression is so slight that it would scarcely be appreciable in ordinary feeding practice.

A more complete and detailed write-up of the experiment will be found in a thesis submitted by the writer for the Degree of Master of Science at the Pennsylvania State College.

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# CALCULATING THE AVERAGE PRODUCTION OF A DAIRY HERD*[1]

R. B. BECKER

*Division of Dairy Husbandry, University of Minnesota, University Farm, St. Paul, Minnesota*

The value of a dairy herd today, is measured not so much by the outstanding records of one or two exceptional cows, but rather by the high average production of the entire herd. One or two good individuals may have been purchased, or may even crop out accidentally by a fortunate mating in a large herd of cows. On the other hand, a uniformly high average production indicates more uniform transmitting ability, and measures the value of the cows as members of a breeding herd.

Wide diversity exists between the methods of calculation used in determining the average production of dairy herds. Some of the methods in practise are fairly accurate. Other methods introduce a factor of error, on account of the mathematical means employed in the calculation. No comparison has been made of these methods to date. When herd records are published in the farm press, the lack of uniformity in their calculation has tended to give rise to some confusion, in comparing those not determined on the same basis.

Several different methods of calculation are in vogue at the present time. It is the purpose of this paper to discuss certain of these methods, and to present a more accurate method, recently devised by the author.

Cow testing associations, county breeders organizations, chambers of commerce, dairy extension workers, and others have found it advantageous in the past to evaluate dairy herds under their jurisdiction, according to the average production of all the cows within the herds. These organizations have used four

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* Received for publication December 15, 1924.

[1] Published with the approval of the Director as Paper No. 511, Journal Series, Minnesota Agricultural Experiment Station.

methods chiefly. These methods are (1) equivalent average, (2) rough average, (3) herd average with its modifications, and (4) cow-month average.

In order to deal with concrete facts, let us consider an actual herd owned by a member of the Sunflower Cow Testing Association, located in northwestern Kansas. Complete production figures for this herd are tabulated. The records are based upon complete daily milk weights, and a one-day butterfat test each month. Feed, breeding and other records were also kept, but have been purposely omitted from the present discussion. When

TABLE 1

| COW NUMBER | AGE | NOTES | DAYS IN MILK | FEEDING DAYS | MILK | FAT |
|---|---|---|---|---|---|---|
| Cows in N herd during the entire year | | | | | | |
| | *years* | | | | *pounds* | *pounds* |
| 2 | | Grade H | 321 | 365 | 10,127 | 408.8 |
| 3 | | Grade H | 347 | 365 | 10,069 | 357.9 |
| 4 | | Grade H | 281 | 365 | 6,452 | 257.7 |
| 5 | | Grade H | 334 | 365 | 7,726 | 307.9 |
| 6 | | Grade H | 303 | 365 | 8,612 | 294.8 |
| 8 | | Grade H | 321 | 365 | 9,027 | 296.7 |
| 9 | | Grade H | 349 | 365 | 11,199 | 439.1 |
| 10 | | Grade H | 324 | 365 | 11,758 | 415.9 |
| 11 | | Grade H | 319 | 365 | 9,468 | 345.7 |
| 17 | | Grade H | 279 | 365 | 6,319 | 237.4 |
| 18 | | Grade H | 202 | 365 | 5,367 | 217.6 |
| D | 5 | Registered H | 334 | 365 | 10,732 | 374.3 |
| E | 5 | Registered H | 312 | 365 | 10,019 | 364.5 |
| J | 7 | Registered H | 303 | 365 | 8,480 | 313.1 |
| Cows in N herd part of the year | | | | | | |
| 1 | | Culled | 133 | 133 | 2,449 | 71.4 |
| 7 | | Culled | dry | 95 | | |
| 12 | | Died | 228 | 228 | 4,552 | 157.8 |
| 16 | | Died | 304 | 304 | 7,132 | 333.7 |
| 19 | | Culled | 123 | 140 | 2,163 | 107.1 |
| 20 | 2 | Heifer | 172 | 172 | 5,002 | 194.4 |
| 21 | 2 | Heifer | 165 | 165 | 4,671 | 158.7 |
| 22 | 2 | Heifer | 158 | 158 | 4,544 | 147.9 |
| 23 | 2 | Died | 67 | 67 | 2,498 | 84.6 |

feed records are used, they are based upon 365 days feed consumption for each lactating cow, dry periods included.

The fourteen cows completing a lactation period during the year show a total production of 125,355 pounds of milk and 4631.4 pounds of butterfat in 4329 days in milk. Nine remaining cows in the herd of producing age, yielded 33,011 pounds of milk, 1255.6 pounds of butterfat during 1350 days in milk. These nine later cows were of milking age, consumed feed in the herd for a total of 1462 days during the fiscal year. However, only eight of these cows were in milk during the association year. These eight cows consumed feed 1367 days after attaining producing age. Heifers were not considered of producing age until after first calving. The herd of twenty-three cows and heifers yielded a total of 158,366 pounds of milk, 5887 pounds of butterfat during 5679 milking days. Thus we have the herd's total production.

### COMPARISON OF METHODS OF CALCULATION

A consideration of the four methods used in the United States in calculating average production of the herd, will now be made, based on data from the above herd.

1. The "equivalent average" method considers the average number of cows milking daily, or monthly, during the year. This figure is secured by dividing 5679 milking days, by 365 days in the year, or 15.8 "cows." Dividing the total production by 15.8 the equivalent average is found to be 10,023 pounds of milk 372.6 pounds of butterfat. Cow testing association records in several states were reported on this basis in the past, using instead the number of months the cows were in milk (omitting the cows dry). This total number of months is divided by 12, to obtain the number of "cows" in milk during the year, when calculating the average production.

Wherein does this method lack?

A casual glance at the records of the individual cows, shows that only four animals in this herd exceeded 372.6 pounds of butterfat. Clearly, the value attained by this method, is an inflated figure which fails to measure the average cow. The theory upon which it is based, is that a "cow" is milked 365 days during the year. This theory is distinctly wrong, and misrepresents the herd to a considerable extent, especially if the cows are relatively heavy producers during short lactation periods.

As an example, let us compare results of two small herds. Two beef cows milking 4500 pounds of milk in lactations of 180 days each, are equivalent to one "cow" producing 9000 pounds of milk in 360 days, or 9125 pounds of milk in 365 days. This herd production may be compared in the same association, with that of a small herd of two grade Holsteins averaging 7500 pounds of milk each in 310 day lactations. The equivalent average production of these two Holsteins calculated by the same method, would be only 8830.6 pounds of milk.

The mathematics is correct, but is the above basis of comparison fair to both herds? Does it truly represent their relative merits as producers? Does it not rather over-estimate the value of both herds, the short-time producers to a greater extent than the persistent cows?

2. The "rough average" production is obtained by dividing the total production of the herd, by the total number of cows within the herd that have dropped calves prior to, or during the year. In other words, the rough average production of these twenty three cows and heifers is 6885 pounds of milk, 256 pounds of butterfat. This method of calculation has more to recommend it than the equivalent average method. However, it overlooks the fact that cows culled from the herd, or dead during the year, did not pass a normal lactation period. Also, heifers freshening near the close of the year were not given a fair complete measure of their producing capacity. The rough average method somewhat under-estimates the true average production of the herd.

3. The "herd average" method considers only cows that have freshened prior to the first of the year, or that passed a complete lactation in the herd during the year. Fourteen such cows in this herd, calculated upon this basis, actually yielded 8954 pounds of milk, 330.8 pounds of butterfat each.

The herd average method approaches nearer to the actual condition existing in this herd than either of the two previous methods. If the herd management could be such that every cow within the herd could be included by this method, the true value of the average herd cow would be known. In most herds, however, heifers calve, cows are purchased or sold, or some members of the herd may die during the year. A fair method of calculating the herd's average production should consider these cows, since they are an integral part of the producing herd at least part of the period.

Some modifications of the herd average method vary, in that they include records of cows that have been in milk an arbitrary part of the year. Some of these modified methods set the standard at eight months (244 days), and others at six months (183 days) in order that any records of production may be considered as one lactation. The modifications now in use in the United States, as taken from a recent survey made by the Committee on Cow Testing Associations, will be mentioned later.

4. The "cow-month" method originated at the University of Minnesota as the result of a conference between Dr. C. H. Eckles, E. A. Hanson and G. A. Pond. This method accounts for all cows, both fresh and dry, in the herd each month of the year. This total number of cow-months for the year, divided by 12, gives the number of "cows" in the herd for the year. On this basis the N herd had eighteen cows, which averaged 8798 pounds of milk and 327.1 pounds of butterfat.

This method closely approaches accuracy. However, it gives a slightly inflated average on account of allowing no dry period for heifers freshening near the year's close. Small inaccuracies arise from the use of even numbers of months in determining the cow-months at the beginning and close of a lactation period.

The cow-month method is adapted to field work in cow testing associations where a comptometer is not available for making calculations.

The cow-month method differs only in minor details from the older Danish method to be considered next.

5. The system of computing average production of a dairy

herd as used in Denmark, was evolved by the State Consulent, Axel Appel, for use by the very first cow testing association in 1895. In the Danish system, the herd average production is based upon the number of days a cow consumed feed during the year. The total feeding days of part-time cows, is divided by 365 days to obtain their equivalent number in full lactation periods. Thus, the herd under consideration will be calculated by the Danish method.

Fourteen cows of producing age passed full lactations during the year, and consumed feed for 365 days each. Of the cows in the herd part of the year only, number 7 was not in milk, and hence is discarded entirely. The eight remaining cows and heifers consumed feed 1367 days during or following lactation, during the fiscal year. Dividing 1367 by 365, gives an equivalent of 3.75 full feeding years, or a total of 17.75 full feeding years for the entire herd. On this basis the average production of these 17.75 cows by the Danish method of calculation, is 8922 pounds of milk, 331.7 pounds of butterfat.

The Danish authorities also calculate the average production of the "normal" cows in each herd. The records used in such a calculation exclude all first and second calf heifers, all cows that have aborted, or not bred normally, all animals in any way not passing a normal cycle of lactation and reproduction during the fiscal year. In other words, it is based upon the production records of normal, healthy, mature cows, having 365 days feed records, and a normal lactation during the fiscal year. It measures the ideal production of the breeding cows under the prevailing management.

The Danish method makes no allowance for any dry period in connection with heifers recently fresh, or cows sold while still in milk. In so far as it does this, the calculated average production by the Danish method, tends to be slightly overvalued.

6. To solve the problem of finding a fair standard of comparison between herds, the author devised a sixth method, here termed the "lactation average" method. This method has been in use in the Kansas state institution herds for the past three

years, and has proved satisfactory. The lactation average method is believed to overcome certain defects of some of the previously described methods. Although not perfect, it approaches accuracy more closely than either the equivalent or rough average methods. It overcomes one objection registered against the herd average method, by including all lactating cows and heifers. Although quite similar to the cow-month and Danish methods, it is based upon the length of the average lactation period, instead of on the number of feeding days or cow-months. It allows for a proportionate dry period with cows milking only a part of the year.

Fourteen cows in the N herd passed complete lactation periods during the fiscal year. They averaged 309 days in milk. Considering 309 days as the length of a normal lactation period in this herd, it is found that the nine cows of producing age in the herd only part of the year, are equivalent to 4.37 cows in milk 309 days. In other words, the herd production for the year represents the work of 18.37 full lactation periods of 309 days each (representing present methods of breeding management in this herd). Based on 18.37 full lactation periods, the lactation average production would be 8621 pounds of milk and 320.5 pounds of butterfat.

The lactation average method, as devised, is not above criticism. It assumes that in this particular herd, the part-time producers would average 309 days in milk. This is only approximately true. The three heifers are credited with the highest part of their lactation period. Two of the cows were culled at the close of their lactation period, offsetting to some extent the three heifers. Three cows died from various causes during the year. One cull was fattened and slaughtered for beef, but was not in milk during the fiscal year.

The six methods of calculating the average production, as illustrated with the N herd, give the results as outlined in table 2.

It is seen from the summary of results obtained by applying these six methods to the same herd, that the first result is distinctly an inflated figure. The rough average method is too greatly influenced by a few animals that are members of the herd only a part of the year. These two methods have gradually been dropped from use, due to the inaccuracies they involve. The herd average, cow-month, and Danish methods show only a small percentage variation, and more closely approach the true figure. The lactation average method, while not perfect, overcomes the small over-valuations arising from not allowing for a dry period with part-time producers. Thus the lactation average, including all individuals, gives the true measure of average production.

A survey made in 1923, brought to light the fact that the equivalent and rough average methods have been practically discontinued from use. Seven states were using the Minnesota cow-month method, with others looking favorably toward it, for use in cow testing association work. Fifteen states employed the herd average method. Five of these states used only full twelve month records, one ten months, five nine months, two eight months, one seven months, and one state used all records even of only four months duration when calculating the average production of a herd. The latter, of course, gradually introduce increasing errors in computations involving part-time production records, by that method.

TABLE 2

*Comparison of results of calculating the average production of herd N by six methods*

| METHOD | COWS | MILK | FAT |
|---|---|---|---|
| | | *pounds* | *pounds* |
| Equivalent | 15.8 | 10,023 | 372.6 |
| Rough | 23.0 | 6,885 | 256.0 |
| Herd (not modified) | 14.0 | 8,954 | 330.8 |
| Cow-month | 18.0 | 8,798 | 327.1 |
| Danish | 17.75 | 8,922 | 331.7 |
| Lactation | 18.37 | 8,621 | 320.5 |

## SUMMARY

1. There is lack of uniformity among methods employed in the United States in calculating the average production of herds in cow testing associations. Some of these methods are inac-

curate. A comparison of the results obtained by these methods, has been made on the production records of the N herd of the Sunflower Cow Testing Association in northwestern Kansas.

2. The equivalent average method gives an inflated valuation which is especially increased in herds of cattle yielding a large flow of milk in short lactation periods.

3. The rough average method is too greatly influenced by cows passing only a part of their lactation period in the testing association's fiscal year.

4. The herd average method (unmodified) excludes cows passing only a part of the lactation period during the fiscal year. Yet these cows were an integral part of the producing herd for a part of the year. Modifications of the herd average method introduce an error increasing with the shorter records accepted as full lactation periods.

5. The cow-month method is practically identical with the Danish method. Slightly larger errors may be incurred through use of months rather than exact days. No allowance is made for any dry period with part-time producers, thus slightly overvaluing the herd average. The percentage of error, however, is small by this method.

6. The Danish method bases the number of cows upon feeding days of lactating and dry cows, using 365 days as the standard. It closely approaches accuracy, though tends to over-value part-time cows.

7. The lactation average method is based upon the average length of lactation of the normal cows in the herd, and calculates the remaining part-time producers according to the normal length of lactation of the herd cows.

8. The four last methods named all approach accuracy. Of these, the lactation average method more nearly represents the normal management of the herd, and the true average production.

Acknowledgement is made of the assistance of Mr. Monroe Coleman who performed the actual work of securing production records in the Kansas state institution herds under the direction of the Kansas State Agricultural College during the past three

years. Also to C. R. Gearhart of Pennsylvania State College, and P. C. McGilliard of Oklahoma Agricultural and Mechanical College, who offered constructive criticism of the methods of calculation.

The Danish method was translated and made available for reference in October, 1924, by Consulent K. M. Anderson of Taastrup, Denmark.

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# GELATIN AS A SOURCE OF BACTERIA IN ICE CREAM*

J. M. BRANNON AND P. H. TRACY[1]

*Department of Dairy Husbandry, University of Illinois, Urbana, Illinois*

Studies have shown that ice cream may be highly contaminated with bacteria. Gelatin, which is used in most commercial ice cream to the extent of about 0.5 per cent, has been considered by some as an important source of contamination. The investigations of Gordon (1), Hammar (2), Ellenberger (3), and Parfitt (4) have shown that the different brands of gelatin vary widely in the number of bacteria they contain.

It was the purpose of this investigation to study the bacteriological condition of gelatin found on the market at the present time (1923–1924) and to determine the importance of this product as a source of contamination of ice cream.

The samples for this study were obtained from eleven leading gelatin manufacturers and distributors and from ice cream manufacturers throughout the state, as well as from a few in Wisconsin and Oklahoma.

The samples from the gelatin manufacturers and jobbers were received in various kinds of containers. It was interesting to note that the container was not a factor in the contamination of the gelatin. Some of the highest, as well as the lowest, counts were obtained on samples that came in sealed envelopes. Likewise, some of the samples which came in such containers as tin cans and sealed card-board boxes were fairly highly contaminated, while others were sterile or nearly so. The gelatin samples obtained from the ice cream manufacturers were secured by send-

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*Received for publication November 15, 1924.

[1] This work was suggested and planned, and samples were secured by P. H. Tracy; the bacteriological work was done by J. M. Brannon. The authors wish to take this opportunity to express their appreciation of the coöperative spirit shown by the gelatin manufacturers and jobbers and the ice cream manufacturers from whom the gelatin samples for this study were obtained. Thanks are also due Prof. M. J. Prucha, of the Division of Dairy Bacteriology, for his constructive criticism of the manuscript.

ing them sterile bottles and sterile tin spoons with which to dip the samples.

When a sample of gelatin was taken for bacteriological study a sterile spoon was used for a sampler. The scale pan was covered with sterile paper on which the gelatin was placed when it was being weighed. One gram of gelatin was added to 99 cc. of sterile distilled water which had been heated to 45°C. so as to accelerate the dissolving of the gelatin.

The medium used for growing the bacteria was made of 1.5 per cent agar, 1 per cent peptone, 1 per cent lactose, and a small amount of beef extract. The plates were incubated for five days at room temperature.

## DATA

### *Bacterial counts on the gelatin samples*[2]

In table 1 will be found the counts made on different grades of eleven brands of gelatin secured from the gelatin manufacturers and jobbers. It will be noted that the price charged for the gelatin is no criterion of the bacteriological condition of that gelatin. The data show that in general the grades within the different brands run fairly uniform in bacterial count. It is of interest to note that the counts on the samples of 8 of the 11 brands run relatively low, the minimum being 0 and the maximum 396,000, the average of the 38 samples being 30,289. The counts on the other brands are high in several cases.

### *Counts on samples received from ice cream manufacturers*

The counts in table 2 are on gelatin samples received from the ice cream manufacturers. These samples were secured from some of the largest manufacturers in Chicago and Milwaukee, from some of the manufacturers in the smaller cities, and from a few of the small manufacturers in the country towns. Here again it will be noted that in general a high gelatin cost does not necessarily mean a low bacterial count. It is also interesting to

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[2] In this discussion, unless otherwise specified, the count on gelatin refers to the number of bacteria per gram of dry gelatin.

TABLE 1

*The bacterial counts on gelatin samples received from the gelatin manufacturers and jobbers*

Bacteria per gram

| PRICE PER POUND | NO PRICE GIVEN | $0.25–.29 | $0.30–.34 | $0.35–.39 | $0.40–.44 | $0.45–.49 | $0.50–.54 | $0.55–.59 | $0.60–.64 | $0.65–.69 | $0.70–.75 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *brand* | | | | | | | | | | | |
| A | | | | | | Sterile | Sterile | | Sterile | 100 | |
| B | | | 396,000 | 201,500 | 250,000 | | 1,700 | 500 | 200 | 8,000 | |
| C | | | | | 500 | Sterile | 100 | Sterile | Sterile | | |
| D | | | | 3,672,500 | 6,100,000 | | 1,062,000 | 338,000 | | | |
| E | | | 5,500 | | | 500 | 20,200 | | Sterile | 1,200 | |
| F | | | | | | 29,500 | 800 | 3,500 | 1,000 | | |
| G | | | | | | | 7,000 | Sterile | 700 | | |
| H* | | 357,500 | 286,400 | 715,000 | 2,697,500 | 2,398,500 | 2,860,000 | 13,000,000 | 3,500 | 1,443,000 | 149,500 |
| | | 298,000 | | 8,125,000 | 3,103,500 | 62,600 | 31,500 | 87,000 | 1,286,000 | 3,500 | |
| | | | | 12,465,000 | 1,449,500 | 42,000,000 | 1,100 | 3,840,000 | | | |
| | | | | | 3,250,000 | | | | 4,000 | | |
| | | | | | | | | | 257,000 | | |
| I | | 7,600 | 9,000 | 20,700 | 83,000 | 3,800 | 20,300 | | 3,400 | 6,800 | 47,600 |
| | | | | | | 12,500 | | | | | |
| J | 2,600 | | | | | | | | | | |
| | 700 | | | | | | | | | | |
| | 3,900 | | | | | | | | | | |
| | 600 | | | | | | | | | | |
| K | 310,000 | | | | | | | | | | |
| | 640,000 | | | | | | | | | | |
| | 1,360,000 | | | | | | | | | | |
| | 600 | | | | | | | | | | |

* Jobber.

TABLE 2

*Counts on gelatins received from ice cream manufacturers*

| BRAND* | PRICE REPORTED (CENTS PER POUND) | BACTERIA PER GRAM |
|---|---|---|
| B-1 | 46 | 1,020,000 |
| B-1 | 55 | 1,080,000 |
| B-1 | 50 | 5,000 |
| B-1 | | 2,100 |
| B-2 | 51 | 103,000 |
| B-2 | 50 | 37,400 |
| B-2 | | 1,000 |
| B-3 | 60 | 165,000 |
| B-3 | | 1,300 |
| B-4 | 65 | 1,700 |
| B-4 | | 2,100 |
| B-5 | | 150 |
| F-1 | 48 | 1,200 |
| F-1 | 55 | 5,000 |
| F-1 | 49 | 1,800 |
| F-1 | 51 | 340,000 |
| F-2 | 60 | 300 |
| J-1 | | Sterile |
| J-2 | | 300 |
| J-3 | 63 | 500 |
| J-3 | | 3,800 |
| L | 42 | 7,000 |
| M | 56 | 2,500 |
| N | 52 | 410,000 |
| N | 42 | 280,000 |
| O | 42 | 2,000 |
| O | 42 | 4,000 |
| P | 51 | Sterile |
| Q | 25 | Sterile |
| R | | 10,100 |
| R | | 7,600 |
| S | | 11,500,000 |
| S | | 250,000 |
| T | | 560 |
| U | | 500 |
| U | | 1,970,000 |
| V | 55 | 1,250,000 |

* The sub-number refers to the grade.

note the relatively large number of low count gelatins recorded in the table. Of the 37 samples, 3 were sterile, 21 had a count between 100 and 10.000, 8 had a count between 10,000 and 500,000, and only 5 had a count above 1,000,000.

### *The uniformity of the bacterial count of gelatin*

To determine the uniformity of the bacterial count of gelatin, samples of some of the brands were obtained at different times. These counts are given in part 1 of table 3. In grade 4, shipment 2, under brand A, will be found a count which is much higher than that on the other samples of this brand. The writers cannot account for this great difference since two other samples of the same grade ran low in bacteria.

In addition, a comparison was made of the counts of gelatin received from the gelatin manufacturers and of the counts of similar brands sent in by the ice cream manufacturers. These counts are given in part 2 of table 3. It will be noted that in a few cases there is a wide variation in the counts, but in general the number of bacteria in the different gelatin samples of the same brands compare favorably. Of course it is not known whether the lots of gelatin were manufactured at the same time or were from different runs.

### *The importance of the gelatin preparation*

Gelatin is added to the ice cream mix in either a dry or dissolved form. The method used depends a great deal upon the plant equipment and the general procedure followed in preparing the mix. When added dry, the gelatin is ordinarily sprinkled into the cold mix before pasteurization. When the gelatin is added in a dissolved form it is usually heated in a container in a hot water bath, in a milk can by the introduction of steam, or in a steam jacketed kettle especially made for the purpose. The temperature to which the gelatin is heated and the time it is held naturally vary among different ice cream makers.

Ellenberger (3) found that the temperature to which the gelatin was heated was an important factor in the destruction of

TABLE 3

*Counts* on different samples of the same brands*

Part 1

| BRAND A | | | | BRAND B | | |
|---|---|---|---|---|---|---|
| Grade | Shipment 1 | Shipment 2 | Shipment 3 | Grade | Shipment 1 | Shipment 2 |
| 1 | Sterile | 200 | 500 | 1 | 396,000 | 500 |
| 2 | Sterile | 500 | 200 | 2 | 210,500 | 900 |
| 3 | Sterile | 250 | 800 | 3 | 250,000 | 12,000 |
| 4 | 100 | 1,130,000 | 500 | 4 | 251,700 | 15,000 |
| | | | | 5 | 500 | 31,000 |
| | | | | 6 | 200 | 38,000 |
| | | | | 7 | 8,000 | 41,000 |

Part 2

| BRAND | SAMPLES FROM GELATIN MANUFACTURERS | SAMPLES FROM ICE CREAM MANUFACTURERS |
|---|---|---|
| F-1 | 3,500 | 5,000 |
| | | 1,200 |
| | | 1,800 |
| | | 340,000 |
| F-2 | 1,000 | 300 |
| B-1 | 1,700 | 1,020,000 |
| | 41,000 | 1,080,000 |
| | | 5,000 |
| | | 2,100 |
| B-2 | 34,000 | 103,000 |
| | 58,000 | 37,000 |
| | 500 | 1,000 |
| B-3 | 12,000 | 1,300 |
| | 15,000 | 165,000 |
| | 200 | |
| B-4 | 900 | 1,700 |
| | 8,000 | 2,100 |
| B-5 | 500 | 150 |
| J-1 | 2,600 | Sterile |
| J-2 | 700 | 300 |
| J-3 | 3,900 | 500 |
| | | 3,800 |

* Number of bacteria per gram.

the bacteria. He reports that the average total count, for the 15 samples of gelatin studied, when dissolved at a temperature of from 125° to 130°F, was 388,162; when dissolved at a temperature of 160° to 170°F. the average was 1172, and when dissolved in boiling water the average count was reduced to 530.

TABLE 4

*Relation of the method of preparing the gelatin to its bacterial count*

Part 1

| DATE PLATED | COUNT PER CUBIC CENTIMETER OF RINSE WATER ADDED TO UNWASHED PAN | TEMPERATURE TO WHICH HEATED | COUNT AFTER BEING HEATED (PER GRAM) |
|---|---|---|---|
| May 21 | | 180°F. | 800 |
| May 22 | | 140°F. | 900 |
| May 26 | 55,000 | 180°F. | 900 |
| May 28 | | 150°F. | 900 |
| June 3 | | 160°F. | 2,450 |
| June 6 | 1,700 | 160°F. | 900 |

Part 2

| DATE HEATED | COUNT PER CUBIC CENTIMETER OF RINSE WATER ADDED TO UNWASHED PAN | COUNT OF RAW SKIM MILK IN WHICH GELATIN WAS TO BE DISSOLVED | TEMPERATURE TO WHICH HEATED | COUNT OF HEATED SKIM MILK WHEN RAISED TO DIFFERENT TEMPERATURE | COUNT PER GRAM OF GELATIN HEATED IN SKIM MILK SOLUTION | COUNT PER GRAM OF GELATIN HEATED IN A WATER SOLUTION |
|---|---|---|---|---|---|---|
| September 19 | | | 140°F. | | | 250 |
| September 20 | 900 | 700,000 | 150°F. | 600,000 | 3,600 | 850 |
| September 23 | 110,000 | | 150°F. | | | 330 |
| September 26 | 28,200 | 10,000 | 160°F. | 3,300 | 700 | 270 |
| September 28 | 5,000 | 480,000 | 170°F. | 2,000 | 1,500 | 210 |
| October 1 | 700 | | 160°F. | | | 170 |
| October 3 | 4,800 | 450,000 | 140°F. | 163,000 | 1,820 | 600 |
| October 6 | 600 | | 160°F. | | | 150 |
| October 10 | 2,100 | | 160°F. | | | 440 |

The counts in column six (6) were obtained by substracting the count on the heated milk from the count secured from heated milk and gelatin.

To determine the importance of the care given the gelatin cooker and the effect of the heating process upon the bacterial count of the gelatin, the following experiments were conducted:

May 21 a liter of a ten per cent solution of a gelatin having a count of 5000 bacteria per gram was heated to 180°F. in a 2-quart aluminum double boiler. The count was reduced to 800 per gram. The gelatin pan was emptied and allowed to remain unwashed at room temperature until the next day when a fresh supply of the gelatin solution was added to the pan and heated this time only to 140°F. The count per gram of gelatin was 900. The operation was repeated and, in addition, the gelatin pan, after standing twenty-four hours unwashed, was first rinsed with 99 cc. of sterile distilled water. This rinse water contained 55,000 bacteria per cubic centimeter (see table 4, part 1, column 2) and the gelatin after being heated contained 900 bacteria per gram. This experiment was continued until June 6, the results being shown in table 4.

Since the gelatin used in the experiment recorded in part 1 of table 4 was low in bacteria, the experiment was repeated using a gelatin which had a count of 6,100,000. The results are given in part 2 of table 4. Part 2 also includes the counts obtained on gelatin dissolved in skim milk.

In another experiment, conducted during the hottest part of the summer, an unwashed gelatin pan stood for forty-eight hours at room temperature, during which time the gelatin liquified. Ninety-nine cubic centimeters of sterile distilled water was added and a count of 1,988,000,000 bacteria per cubic centimeter was obtained from the rinse water. Then a ten per cent gelatin solution was added to the pan and heated to 166°F. and the gelatin plated. The count secured was 6180 per gram (the original gelatin containing 300 bacteria per gram) an increase of nearly 2000 per cent in the count.

Although it might be considered desirable from a bacteriological standpoint to heat the gelatin solution to a high temperature, it should be remembered that too high a temperature is likely to injure the jellying qualities of the gelatin. The effect of increased temperature upon the strength of a 3.5 per cent solution of high grade gelatin is shown in table 5. The samples were prepared by dissolving the gelatin at 125°F. and then adding it to tin cans which after being sealed were heated in hot water at the indicated temperature for thirty minutes, taking about ten minutes for the water to reach that temperature. The cans were then removed to the 40°F. storage room and held for a period of fifteen hours. They were then held at room temperature for six hours, when they were opened and the jelly strength determined by means of a tester of the type used by the Forest Products Laboratory at Madison, Wisconsin.

While the above relation of temperature to jelly strength probably would not hold for all gelatins it shows the tendency for the higher temperatures to weaken the strength of the gelatin. For this reason the gelatin solution should not be pasteurized at too high a temperature.

TABLE 5

*Relation of temperature to jelly strength*

| TEMPERATURE | JELLY STRENGTH (DEPRESSION IN CENTIMETERS) |
|---|---|
| 140°F. | 1.725 |
| 160°F. | 1.75 |
| 170°F. | 1.80 |
| 180°F. | 1.85 |
| 190°F. | 1.925 |
| 200°F. | 2.65 |
| Boiled | Too weak to measure |

Autoclave—12 pounds pressure 50 minutes—liquid-like water.

### *Relation of condition of storage to the number of bacteria in gelatin*

To determine the importance of the condition under which gelatin is held, six Sealright pint containers were filled with gelatin and stored under the different conditions indicated in table 6. The samples were placed April 16 and counts were made at different intervals between that date and October 24. The difference in the humidity of the rooms in which the gelatins were stored resulted in a difference of moisture content as shown in the table.

## DISCUSSION

The data presented in tables 1, 2 and 3 indicate that it is possible to manufacture gelatin that is practically sterile. Twelve of the 142 samples examined were free from germs. In general

TABLE 6

*Relation of the condition of storage to the bacterial count*

| DATE OF COUNT | COUNT |
|---|---|
| A. 0° to 10°F. storage room (dry) | |
| May 1 | 1,400 |
| June 12 | 1,300 |
| July 15 | 2,400 |
| September 1 | 1,700 |
| October 24 | 800 |
| Percentage moisture September 13 = 13.5 | |
| B. 90° to 100°F. storage room (dry) | |
| May 3 | 700 |
| May 23 | 300 |
| June 12 | 450 |
| July 15 | 1,200 |
| September 1 | 500 |
| October 24 | 550 |
| Percentage moisture September 13 = 12.05 | |
| C. 30° to 40°F. storage room (damp) | |
| May 1 | 1,000 |
| May 23 | 1,500 |
| June 12 | 1,500 |
| July 15 | 1,000 |
| September 1 (sample had become lumpy) | 4,000 |
| October 24 | 41,000 |
| Percentage moisture September 13 = 30.22 | |
| D. 30° to 40°F. storage room (in desiccator) (dry) | |
| May 1 | 3,400 |
| May 23 | 4,200 |
| June 12 | 2,000 |
| July 16 | 4,800 |
| September 1 | 3,700 |
| October 24 | 1,000 |
| Percentage moisture September 13 = 11.76 | |
| E. 65° to 75°F. storage room (damp) | |
| May 23 | 2,400 |
| June 12 | 500 |
| July 15 (sample had become lumpy) | 10,000 |
| September 1 | 17,700 |
| October 24 | 115,000 |
| Percentage of moisture September 13 = 25.09 | |

TABLE 6—*Continued*

| DATE OF COUNT | COUNT |
|---|---|
| F. 85° to 100°F. storage room (dry) | |
| May 1 | 5,000 |
| May 23 | 2,000 |
| July 16 | 3,700 |
| September 1 | 700 |
| October 24 | 700 |
| Percentage moisture September 13 = 12.64 | |

the samples ran low in bacteria, since fifty per cent of them contained less than 20,000 bacteria per gram, whereas only thirty-one per cent (nearly one-half of which were of the same brand) contained over 100,000 bacteria per gram. It is apparent that many of the gelatin manufacturers are producing gelatin of low bacterial count.

Samples of some of the brands ran uniformly low in bacteria while the samples of other brands were much more highly contaminated. It might be inferred from this that the method of manufacture is a very important factor in the number of bacteria present in gelatin.

The heating of gelatin to 140° to 160°F. greatly reduces the number of bacteria present, even though the gelatin may be highly contaminated. The efficiency of pasteurization seems to be greater in a water solution than in a skimmilk solution.

It is evident that neglecting to clean the gelatin cooker after it is used will increase the bacterial contamination from gelatin. The importance of this factor depends somewhat upon the temperature and humidity of the room in which the gelatin cooker is placed.

It does not seem likely that the bacterial count of gelatin increases to any extent after the gelatin leaves the manufacturer unless it becomes damp or is contaminated by some foreign material. Some observations by the writers confirm this opinion. There were in the laboratory two uncovered kegs of gelatin. In April counts were made on both of them. One had a count of 300 and the other had a count of 5000. During the summer several counts were made of the first gelatin and each time it gave a count of 300. Three counts were made of the second gelatin obtaining 5000, 8000, and 5000 bacteria per gram, respectively. The first of these counts was made in April, the second in July and the last in September. These kegs were kept in a dry place. The results in table 6 also suggest that there is not much change in the bacteria content of the gelatin if it is kept dry.

From the data obtained in this investigation the writers conclude that the addition of gelatin to commercial ice cream results in a very slight increase in the number of bacteria.

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# THE EFFECT OF SEASON ON THE MILK AND FAT PRODUCTION OF JERSEY COWS*

C. ELMER WYLIE

*Department of Dairying, University of Tennessee, Knoxville, Tennessee*

It is a well established fact that the milk flow of a cow tends to decrease as the period of lactation advances and that the per cent of fat in the milk tends to increase during the same period. Recent investigations have shown that the season of the year also has an important relation to the amount and richness of milk which a cow produces. The work of Eckles (1) Erf and Cunningham (2) Ragsdale and Turner (3) and Wylie (4) shows that there is a tendency for the percentage of fat in cows' milk to be low in summer and high in winter.

It is important therefore to determine the combined effect of the period of lactation and the season on the milk production of cows. It is the purpose of this paper to show how season affects the production at the same time making a true allowance for period of lactation. The records used in making this determination were more than 2900 individual Register of Merit Records of Jersey cows furnished by the American Jersey Cattle Club for cows freshening in the year 1921 throughout the United States.

Figure 1 shows the average daily production of milk for all cows regardless of the time they freshened. This shows that the high production of milk was 27.2 pounds daily average for June and that this gradually declined to November with an average daily production of 22.8 pounds of milk. From November there was a gradual incease to June.

Table 1 shows the average per cent test of fat by months for cows according to the month in which they freshened. It will be noted that the cows which freshened in January averaged 4.89 per cent fat for that month. There was a gradual increase

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* Received for publication October 25, 1924.

from this until the sixth to eighth month when there was only slight variation followed by a rise at the end of the lactation period. The cows which freshened in June changed but little

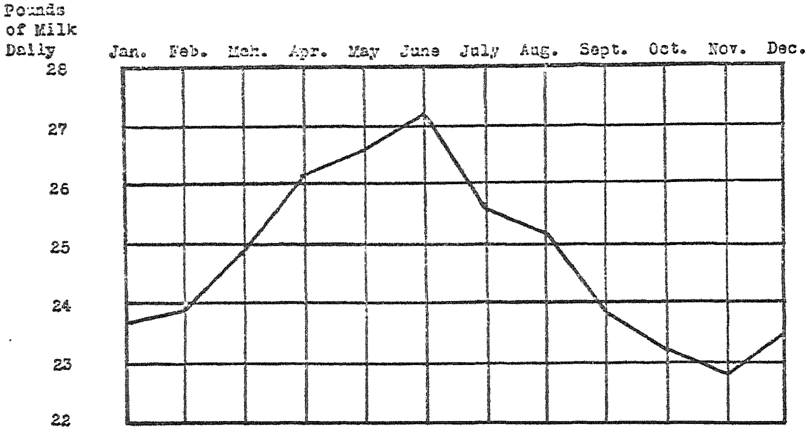

FIG. 1. SEASONAL VARIATION IN MILK PRODUCTION

Table compiled by Paul Reaves, senior student in Dairying, 1924, University of Tennessee.

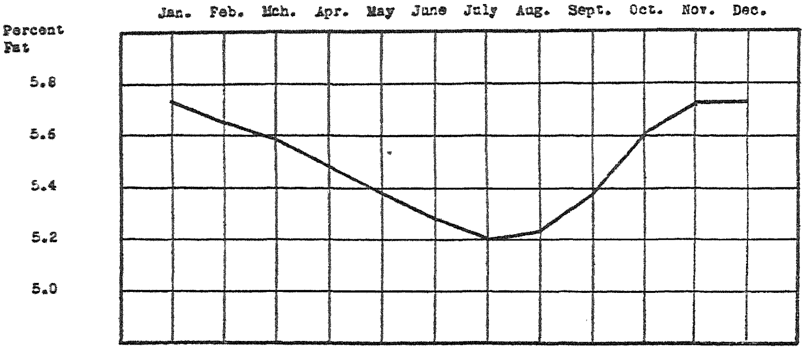

FIG. 2. SEASONAL VARIATIONS IN BUTTERFAT TESTS OF JERSEYS

Table compiled by T. G. Hinton, senior student in Dairying, 1924, University of Tennessee.

after the sixth month. The cows which freshened in July tested highest in the seventh month or January. In practically all other cases the highest tests occurred at the end of the lactation period.

TABLE 1[*]

*Average per cent of fat according to month in which cows freshen*

| MONTH COW FRESHENED | NUMBER OF COWS | MONTH OF LACTATION | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| January | 326 | 4.89 | 4.93 | 4.95 | 5.06 | 5.20 | 5.07 | 5.07 | 5.15 | 5.44 | 5.73 | 5.93 | 5.90 | 5.95 |
| February | 323 | 4.71 | 4.93 | 4.96 | 5.03 | 5.06 | 5.03 | 5.10 | 5.40 | 5.70 | 5.96 | 5.81 | 5.96 | 5.80 |
| March | 326 | 4.88 | 4.93 | 5.03 | 5.07 | 5.06 | 5.29 | 5.51 | 5.89 | 6.07 | 6.16 | 6.19 | 6.27 | 6.21 |
| April | 330 | 4.84 | 4.86 | 4.89 | 4.89 | 5.07 | 5.36 | 5.71 | 5.90 | 5.95 | 5.98 | 5.97 | 6.02 | 6.03 |
| May | 323 | 4.76 | 4.76 | 4.82 | 4.97 | 5.22 | 5.72 | 5.88 | 5.93 | 6.05 | 5.97 | 5.91 | 5.85 | 5.71 |
| June | 225 | 4.63 | 4.63 | 4.87 | 5.15 | 5.57 | 5.73 | 5.82 | 5.88 | 5.86 | 5.86 | 5.80 | 5.84 | 5.93 |
| July | 125 | 4.73 | 4.76 | 5.07 | 5.46 | 5.75 | 5.89 | 6.03 | 6.01 | 5.93 | 5.85 | 5.87 | 5.82 | 5.86 |
| August | 151 | 4.65 | 4.79 | 5.15 | 5.48 | 5.71 | 5.78 | 5.74 | 5.83 | 5.72 | 5.64 | 5.64 | 5.68 | 5.88 |
| September | 211 | 4.79 | 5.01 | 5.28 | 5.60 | 5.76 | 5.79 | 5.74 | 5.64 | 5.63 | 5.50 | 5.57 | 5.75 | 5.89 |
| October | 197 | 4.95 | 5.11 | 5.32 | 5.63 | 5.68 | 5.61 | 5.58 | 5.60 | 5.47 | 5.55 | 5.61 | 5.86 | 6.16 |
| November | 200 | 5.01 | 5.13 | 5.35 | 5.52 | 5.52 | 5.51 | 5.43 | 5.41 | 5.38 | 5.52 | 5.79 | 5.87 | 6.29 |
| December | 167 | 5.07 | 5.13 | 5.25 | 5.36 | 5.45 | 5.46 | 5.38 | 5.39 | 5.43 | 5.78 | 6.05 | 6.21 | 6.27 |

[*] Table compiled by T. G. Hinton and H. W. Arnold, senior students in Dairying 1924, University of Tennessee.

TABLE 2[*]

*Average yearly milk and fat production according to month in which cows freshen*

| MONTH COW FRESHENED | NUMBER OF COWS | MILK | FAT | FAT |
|---|---|---|---|---|
| | | *pounds* | *per cent* | *pounds* |
| January | 325 | 8857.86 | 5.30 | 470.15 |
| February | 337 | 8859.84 | 5.31 | 471.13 |
| March | 325 | 8828.65 | 5.35 | 472.80 |
| April | 331 | 8700.05 | 5.31 | 462.61 |
| May | 323 | 8787.00 | 5.33 | 468.79 |
| June | 225 | 8759.16 | 5.39 | 472.92 |
| July | 125 | 8952.89 | 5.47 | 489.97 |
| August | 151 | 8566.80 | 5.39 | 462.08 |
| September | 211 | 8652.97 | 5.45 | 472.35 |
| October | 196 | 8825.85 | 5.45 | 481.28 |
| November | 200 | 8900.29 | 5.38 | 479.40 |
| December | 167 | 8853.18 | 5.38 | 477.11 |
| Average | | 8796.12 | 5.39 | 473.89 |

[*] Table compiled by T. G. Hinton and H. W. Arnold, senior students in Dairy 1924, University of Tennessee.

Figure 2 shows the average per cent of fat for all cows for the various months, regardless of the time they freshened. The highest tests were for November to January inclusive with an average of 5.73 per cent fat. There was a very gradual decline in test from January to July the lowest month with an average of 5.2 per cent fat and this was followed by a more rapid increase in November.

Table 2 shows the yearly production of cows freshening at the various months of the year. The cows which freshened in January averaged 8857 pounds of milk containing 470 pounds of fat with an average test of 5.30 per cent. The highest milk production was for the cows freshening in July with an average of 8952 pounds containing 489 pounds of fat with an average of 5.47 per cent fat. November was the next highest in milk production but October was the second highest in fat production. The lowest production was 8566 pounds of milk containing 462 pounds of fat with an average test of 5.39 per cent fat for cows freshening in August. There were 337 cows which freshened in February, the largest number for any month while the lowest was 125 for July.

The cows which freshened in January averaged 5.30 per cent the lowest, while the cows which freshened in July averaged 5.47 per cent fat the highest for the entire lactation period. The increase in test of the latter was 0.17 per cent fat higher than the lowest month. The difference between the high and low months in milk production was 386 pounds. The difference between the high and low months in fat production was 27 pounds.

## SUMMARY

A study of more than 2900 Register of Merit Records of Jersey cows showed the following:

1. The cows which freshened in May and July gave the richest milk on the average in the ninth and seventh month respectively of the lactation period. The cows which freshened in June averaged a higher test of fat for the eighth to tenth month in period lactation than they did in twelfth month. The average per cent of fat in the milk, for a year for cows freshening in July, September, and August was the highest, being at least 5.45 per cent.

2. The yearly milk production was highest for the cows freshening in July, October, November, December, January, February, and March, all being over 8800 pounds. April, May, June, August, and September were the low months, although on the average the difference between the two groups was not great.

3. The yearly fat production averaged the highest for cows which freshened in July, October, November, and December, all being above 477 pounds of fat. April, May, and August were the low months all being below 468 pounds of fat. The cows which freshened during the month of July averaged 27 pounds of fat more than those which freshened in April and August.

4. The season of freshening as well as the period of lactation affects the richness of milk from a cow. The richest milk may be produced at some time before the end of the lactation period depending on the month in which the cow freshened.

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# TREATMENT OF CREAM FOR THE REMOVAL OF OBJECTIONABLE FLAVORS AND ODORS[1]

O. F. HUNZIKER

*Blue Valley Creamery Company, Chicago, Illinois*

## IMPROVEMENT OF QUALITY OF CREAM RECEIPTS MOST FUNDAMENTAL

In discussing the subject of removing objectionable flavors and odors from cream, it should be understood at the outset that whatever treatment is given the cream in the factory is not meant to excuse or condone the production of cream of poor quality, nor are such efforts to remove flavor defects offered as a substitute for efforts of cream improvement on the farm through greater cleanliness, better care, and more frequent delivery by the producer. There is no substitute for the production of better cream.

Poor quality of cream is the curse of the industry and all efforts to eliminate its production should be encouraged by every means at our disposition. The relatively small volume of inferior cream and the general good quality of the cream receipts last spring and early summer gave promise that last year's efforts of the creamerymen at cream-grading and quality-paying were beginning to show concrete results in the form of better cream. During the hot weather months, however, the quality of the cream in the southern tier of the middle-west suffered a most discouraging slump. This suggests that the cool weather early in the season was probably largely responsible for the good quality of cream received at that time and that the difficult task of permanent cream improvement is by no means solved yet and still demands the concentrated effort of all agencies interested in the quality of American butter.

Our greatest drawback in the middle-west is the relative scarcity of cow population. In large areas of the corn belt there are but few cows to the farm and the milking of cows is a mere side-line of general farming. A few cents more for better cream does not mean much in farm income to the farmer who has but little cream to sell. The difference in market price between good and poor butter is not sufficient to induce him to keep obnoxious weeds out of his pastures and to divert more time from his general and principal farm work to extra care and more frequent delivery of his cream, in order to secure the top price from the creamery that buys cream on the quality basis. This applies especially wherever coöperation among competing creameries is lacking and where the farmer, therefore, can find a profitable market for his cream regardless of inferior quality.

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[1] Address at Annual Meeting of American Dairy Science Association.

In the more highly developed dairy sections, as in Minnesota, Wisconsin, parts of Iowa, and the Pacific Coast States, conditions for cream improvement are far more favorable and the possibilities for cream of uniformly good quality are practically assured. The herds there are larger and contact between creamery and patron is more direct and closer. The farmer is a dairyman whose dominating business is to milk cows; he produces a larger quantity of cream and every additional cent he can get for cream of better quality means a worthwhile increase in his farm income. It pays him to spend the extra time and labor necessary to meet the requirements of the creamery that pays for quality.

There is no question that if all creameries paid for cream on the quality basis cream improvement would be an established fact. One hundred per cent coöperation among competing creameries would wipe out the only profitable market available for cream of poor quality. The producer then would have to produce better cream or quit selling cream altogether. This ideal is sound but, so far, it has fallen short of successful, practical application, particularly in the corn-belt states.

### MUST MAKE BEST OF EXISTING CONDITIONS

I repeat that the fundamental efforts toward permanent improvement of American butter must ever be centered on means to *prevent* the production of inferior cream. Our organization has been, is now, and will continue carrying on a definite plan of cream-grading and quality paying, both in the form of paying a special price over and above our standard quotations for sweet cream of good flavor and by penalizing the very poorest cream.

Our experience in this work, while hopeful, convinces us of the slowness of permanent improvement under existing competitive conditions. We realize that, even at best, off-flavored cream is likely to continue to arrive at and be received by the creameries. It will be with us for some years to come. We must, therefore, resort to remedies as well as means of prevention; remedies that will pick up the threads of cream improvement where inadequate prevention has dropped them. We must try to undue the defects which it was beyond our control to prevent, as far as such remedy is physically possible and consistent with pure food laws and regulations. We must avail ourselves of every legitimate means that science and practical experience provide to remove such objectionable flavors and odors as may be present in the cream at the time we receive it.

## CLASSIFICATION OF OFF-FLAVORS IN CREAM

The ease or difficulty with which objectionable flavors and odors may be removed from cream varies greatly and depends principally on their origin. It seems logical, therefore, for the purpose of this discussion, to group the multitude of flavor defects according to the causes that produce them.

*1. Feed and weed flavors.* The most prominent among these, from the standpoint of their detrimental effect on the market value of the butter are, wild onion, garlic, leek, and French weed. Other pronounced, though less intense off-flavors of this group are sweet clover, turnip, mustard, potato, etc. Most of these feed and weed flavor defects are local and seasonal. While they do not appear everywhere, nor the year around, they are very common in certain localities and appear at their chosen season with exasperating regularity. While they last they are a menace to the industry, often dropping the market value of butter that is impregnated with them to a point that means heavy financial loss to the creamery.

*2. Flavors absorbed from surroundings.* These flavor defects are not so common. They appear spasmodically and generally only in isolated cases, but their presence in butter is very objectionable. Among the better known of these are oily, gasoline, kerosene, and diverse vegetable flavors. They usually occur where cream is stored in close proximity to materials of which they are an inherent part. The oily flavor is often traced to faulty conditions surrounding the operation of the cream separator as, for instance, the use of too much separator oil around the bearings and spindle and operating the machine without having the bearing guards in place. This frequently causes the oil to be plastered over the separator frame and the nearby wall, saturating the air with the odor of separator oil, for which odor the cream appears to have a marked affinity. The operation of a gasoline engine in the separator room without proper exit for the exhaust will give the cream, almost invariably, a gasoline flavor. The storing of the cream in the basement, or similar places on the farm, where vegetables are kept or potatoes are sprouting, usually impregnates such cream with the respective vegetable flavors and odors, etc.

*3. Flavors due to contamination of cream with foreign substances.* These cases of off-flavors are purely local but their occurrence at times is menacing and costly to the creamery. Among these cases, gasoline, kerosene, and metallic flavors are the most common. Investigation shows that occasionally the railway station agent or the farmer himself is using the empty cream shipping cans as a carrier of gasoline and sometimes of kerosene and remnants of these liquids are left in the can at the time it is filled with cream. The use of milk utensils, such as dippers and pails, for transferring kerosene has also been found responsible for kerosene-flavored cream in some instances, etc. Metallic flavor is often attributable to rusty cans, but has also been found to result from bacterial contamination.

*4. Flavors due to bacterial contamination and fermentation.* This obvious, well-known and very common source of flavor defects leads to a great variety of off-flavors, the type and intensity of which are largely controlled by the relative standard of cleanliness of cream production, the temperature at which the cream is held on the farm and in transit, and the age of the cream when it reaches the creamery. The most common individual flavors of this group are, stale, cheesy, metallic, yeasty, bitter, high acid. It is not necessary here to discuss cause and effect of this group; the subject is well known to all of you, except possibly to emphasize that age is the greatest factor of them all, as it intensifies the detrimental effect of any condition adverse to good flavor to which the cream may have been exposed.

## DEVELOPMENT OF METHODS TO REMOVE OBJECTIONABLE FLAVORS AND ODORS FROM CREAM

For many years the possibilities of freeing cream intended for butter making from flavor defects have been subjected to experimentation and study. The records of the patent office show numerous patents granted on equipment and methods to improve the flavor of milk and cream. The fundamental principle of most of these inventions has to do with exposure of milk and cream to air either by passing air over thin layers of the liquid or by blowing air through the body of the liquid, and in some of the later processes the treatment has shifted from air treatment to vacuum treatment. The temperature of the cream during treatment varies with each invention ranging anywhere from room temperature or lower to the boiling point of water. Most of these designs and processes have merit and each one of them would probably be helpful in removing at least a limited portion of off-flavors present. But in the face of our own experience in trying out the possibilities and limitations of flavor-removing apparatus and processes, we are forced to conclude that most of these inventions are entirely inadequate to remove from cream, completely, such intense and stubborn off-flavors as wild onion, garlic, and the like. Furthermore, the mechanical phase of many of these inventions precludes their adaptability for treatment of commercial cream intended for butter making. It is probably for these and similar reasons that the great majority of these inventions have never passed beyond the stage of theoretical patent designs and hypothetical descriptions and that they have failed to receive the serious consideration of the industry for which they were obviously intended.

You may recall that about fourteen years ago a wave of blowing cream with air swept the middle-west. Creamery supply houses placed on the market complete units for blowing air through the cream in cream-ripening vats and the use of these units became very popular. It took several years of practical experience to establish the fact and to convince the users that the results obtained with that equipment were disappointing and did not justify the expense of its installation and operation. The principle of aeration was sound enough but the method applied was utterly inadequate and incapable of removing enough of the objectionable flavors to accomplish visible improvement of the quality of the resulting butter.

In 1914 Ayres and Johnson (1) published experimental results on removing onion flavor from sweet milk and sweet cream. While their experiments were confined to sweet milk and sweet cream only and conducted on a laboratory scale, their work was a vast step in the right direction. They found that the successful removal of onion flavor required far more intensive air treatment than had heretofore been employed.

In 1920 the market was flooded with an exceptionally large volume of butter with garlic and wild onion flavor. This caused an unprecedented slump in the market value of butter containing these strong off-flavors. Onion-flavored butter sold at prices as low as twenty cents under Extras. It was at that time that the Blue Valley Research Laboratory undertook an extensive investigation, the purpose of which was to subject all onion and garlic-flavored cream to such treatment as would make possible the manufacture of butter free from these disastrous flavor defects. Our treatment consisted of passing heavy currents of filtered air through a spray of cream in several directions for a prolonged period of time. The cream was kept hot by recirculating it continuously through a flash pasteurizer. Before treatment the cream was standardized to a definite point of acidity to forestall serious interference from abnormal curdling during treatment.

Our early experiments demonstrated that it was possible to remove every vestige of onion flavor from sour cream by continuing the treatment long enough. But we soon came to realize that to subject commercial cream to such violent treatment involves several complex and seemingly evasive physical and mechanical problems that affected the texture of the resulting butter. The chief difficulty was the tendency of the cream to churn in the vats and the appearance of a pronounced mealy texture in the butter. Using heated air in the place of recirculating the cream through the flash pasteurizer and adopting a practical system of flash-cooling materially reduced these tendencies.

This system was in operation successfully for two years in one of our factories and for one year in two more factories. It not only did away with onion-flavored butter but it proved very helpful in improving cream with other off-flavors.

Its greatest drawback was the time required for treatment and the constant danger of jeopardizing the texture of the butter. Our efforts, therefore, were directed towards a modification of this process, that would produce equally good results in removing off-flavors in a shorter time and thereby insure with greater certainty the preservation of normal texture of the butter. These efforts led to the conception of operating the air treatment in a partial vacuum confined in a glass-lined container. So, in conjunction with the Pfaudler Company of Rochester, N. Y., an apparatus was developed to accomplish this. This apparatus is now manufactured and sold by the above company under the trade name "Pfaudlerizer" and both apparatus and process are covered by patents held by the Pfaudler Company. The Pfaudlerizer consists of a jacketed vacuum pan, constructed of glass-lined steel, equipped with an arrangement for breaking the cream up into a fine spray and with fittings that make possible the recirculating of the cream during treatment. It has an intake also for the heated air and an impeller for agitating the cream.

### OPERATION OF THE PFAUDLERIZER

After standardizing the cream for acidity and adding to it an amount of water approximately equal to that evaporated during treatment, the cream flows through flash pasteurizers and enters the vacuum pan in the form of a fine spray. While the cream enters the pan a vacuum of from 20 to 24 inches is maintained. When the cream is all in, it is caused to circulate by pumping it from the bottom of the pan back through the jet in the top. At the same time, heated air is blown into the pan in sufficient quantities to reduce the vacuum to about 7 inches while the vacuum pump is in continuous operation. When all of the removable flavors are expelled, as is readily determined by taking samples of cream at intervals, the circulation is stopped, the air blast is shut off, and the vacuum is once more raised to 20 inches or above in order to remove excess air.

The vacuum is then broken and the cream is pumped through a flash cooler into the holding tank. Our aim is to have it enter the holding tank at as near churning temperature as possible so as to obviate the necessity of prolonged cooling and agitation. The time of treatment depends entirely on the quality of the cream. Cream with only slight off-flavors is held in the pan for a short time only, or is flashed into and through the pan and is pushed through the cooler into the holding tank in one continuous operation. In the case of garlic cream and cream pregnant with other intense off-flavors, it is given the circulating treatment in the presence of the air blast as long as is necessary in order to accomplish maximum improvement. This may be from 15 to 30 minutes or even slightly longer.

### EFFECT OF TREATMENT ON FLAVOR

It should be understood that this process is capable of removing flavors only that are volatile or volatilizable under the conditions of operation. Many of the most objectionable weed and feed flavors are of this nature, and as previously stated, such off-flavors as wild onion, garlic, and similar weed and feed flavors, are removed completely so that not a vestige of them is detectable in the butter. Our experience has demonstrated that much of the weed-flavored cream is otherwise good cream and when these flavors are removed a very good piece of butter results.

Flavors absorbed by the cream from outside environment, such as oil, gasoline, and diverse vegetable flavors, as explained under off-flavors belonging to group 2, also yield successfully to this treatment, but when gasoline and kerosene flavors are due to gasoline or kerosene having been added to cream, accidentally or otherwise, as discussed under off-flavors in group 3, their complete removal is more difficult.

Fermentation flavors that are volatile are removed, but in most cases of advanced fermentation the production of volatile flavors is accompanied by the formation of other, non-volatile flavor products. These non-volatile off-flavors do not respond to this treatment nor to any other treatment known. What has been torn asunder by the relentless tooth of nature's processes cannot be reassembled and returned to its original state by mere mechanical juggling. Fermentation that means a partial breaking down of the fats or proteins of milk and cream places these products beyond the saving ingenuity of the science of buttermaking. It is for this reason that such off-flavors as rancid, cheesy, old yeasty, bitter, metallic, cannot be removed entirely. The volatilizable portions will escape easily enough, but there remains a residue of objectionable flavors that cannot be removed completely, and that is carried with the cream into the butter.

It is not possible, therefore, to make an Extras from cream that scores Seconds, but it is possible to raise the score of No. 2 cream, improve the flavor of No. 1 cream, decrease the percentage of No. 2 cream, and increase the percentage of butter scoring 90 points and better.

I realize that results expressed in so general terms as the above are somewhat disappointing and that you will logically ask the question, how many points in score is the butter improved by giving this treatment to the cream? This question is somewhat difficult to answer. The specific improvement varies greatly with the particular character of the off-flavor present in the original cream and a definite answer that shows direct results can only be given by making split churnings, treating one-half and not treating the other half of cream from the same batch.

It is somewhat cumbersome and materially interfering with the efficiency of operation to make split churnings in the routine of commercial manufacture. For this reason data of such direct comparisons are somewhat scarce. In an experiment of seven split churnings or fourteen churnings in all, where we used all the cream received and for the sake of the experiment did not grade out any of the poor cream, the treated cream butter scored 90.3 points while the untreated cream butter scored 89.01 points, a difference in favor of the treated cream butter of 1.29 points. These scores represent the averages of three judges scoring this butter independently and without any knowledge of the origin of the packages before them. Our records of the scores of No. 2 butter show that the factories using the Pfaudlerizer are putting out a butter from 1½ to 2½ points higher in score than they did prior to the use of this equipment and are no longer producing onion-flavored butter. In short, then, this treatment eliminates from cream volatile and volatilizable flavor defects such as weed flavors, absorbed flavors, and flavors produced during the early stages of fermentation.

Such results are not attainable by merely passing currents of air through the body of the cream nor by flashing cream into a vacuum alone. Both of these methods of treatment are capable of removing flavors to a limited extent, but from the standpoint of visible improvement of quality, the results are disappointing. For maximum results and complete removal of such stubborn off-flavors as onion and garlic flavor and the like, the treatment must be more intense, the air must come in contact with a larger surface of the cream and the odor-laden air must be removed quickly and completely. The heat of the cream must be maintained at a temperature at which the objectionable flavors most readily volatilize. This combination of conditions is provided in the apparatus and process above described.

## EFFECT OF TREATMENT ON TEXTURE OF BUTTER

The successful removal of flavors and odors present in cream involves the solution of some interesting physical problems. There is a tendency for the cream that has been subjected to this process to churn in the vats and for the resulting butter to have a disagreeable, mealy texture. These defects can be readily and completely prevented by a correct understanding of the physical effect on the fat globules of prolonged exposure of the cream to high temperature and vigorous agitation. While in the early development of this process these difficulties presented a real menace, they have ceased being an important factor; mealiness and premature churning are now a thing of the past.

Scientifically the physical phenomena that enter into this condition require further study. Microscopic examination of the cream at different stages of the treatment show a progressive increase in the size of the fat units. The small fat globules become fewer in number and there is a distinct increase in the size of the average globule. This change begins in the pasteurizer and continues in the vacuum pan. After thirty to forty minutes treatment, the diameter of the average fat unit is many times as large as it was before treatment. The diameter of some of the fat globules increases to the size of 50 to 60 microns and some of the largest fat units show a contorted periphery.

Whether this condition is due to the prolonged exposure to pasteurizing heat, or to prolonged violent agitation of the heated cream, or both, and to what extent the oiling-off tendency of the hot samples of treated cream while waiting for microscopic examination mars the accuracy of these examinations and interferes with the correct interpretation of the results, have not as yet been definitely determined.

It is of interest here to note that Rahn and Mohr (2), who studied the distribution of fat in pasteurized cream, also find a distinct increase in the size of the units of fat in cream due to flash pasteurization. They ascribe the cause of this phenomenon to the agitation of the cream in the pasteurizer, but their report fails to show what would have happened at the same pasteurizing temperature without agitation or with the same agitation at lower temperature.

Our results and observations are clear on the fact that when cream that has been subjected to this violent agitation in the presence of heat, is permitted to lie undisturbed before cooling or is cooled very slowly, the butterfat oils off copiously and butter made from the cream when in this condition has invariably a very mealy texture. If the cooling is done in the presence of prolonged and vigorous agitation the cream will churn before it can be transferred to the churn.

Further study is needed to justify a conclusive explanation of the underlying causes of these results. In the meantime, these findings suggest that the dominating forces that cause normal cream to represent an emulsion of fat in hydrated colloids (skimmilk) are disturbed. These forces are surface tension and adsorption. The equilibrium of the fat globules seems to be weakened, their surface tension diminished and the adsorbed material of concentrated skimmilk on the surface layer of the fat globules physically altered. As the size of the fat globules increases, their surfaces greatly decrease in proportion to their cubic contents, until finally a point is reached where the surfaces of the fat globules are no longer sufficient to hold the emulsion. The emulsion approaches the breaking point, hence the much increased tendency of the cooled cream to churn prematurely.

This oiling-off of the heated cream and its invariable production of mealy butter can be avoided readily by not allowing the treated cream to assume a state of rest until after cooling below the congealing point of the butterfat, which is 70°F. or below, and by cooling to or below this temperature rapidly. The premature churning is guarded against with a certainty by flash-cooling with but little or no mechanical agitation, as is readily accomplished by the use of coolers of the internal tube type.

## SUMMARY

1. The key-note to permanent improvement of the quality of American butter lies in better cream. Those sections in this

country and those foreign countries that have established a reputation for butter of superior quality are invariably also the sections and countries that foster the production of good cream on the farm. Butter is no better than the cream from which it is made.

2. In sections where the herds are small and limited to but a few cows, economic conditions seriously handicap the establishment of conditions essential to the production of cream of high quality. This renders progress in cream improvement slow. Cream from such sections often contain diverse off-flavors. Among these flavor defects seasonal feed and weed flavors, flavors absorbed from foreign substances, and fermentation flavors of varying degrees of intensity are of common occurrence.

3. Many of these off-flavors are volatile or volatilizable. It is possible, by intensive treatment of this cream, to remove the volatile and volatilizable flavors and odors and to thereby improve the quality of the cream and raise the score and market value of the resulting butter.

4. The treatment that has been found most effective in removing off-flavors from cream for buttermaking consists briefly of standardizing the cream to a reasonably low, definite point of acidity, flash pasteurizing it and spraying it into a high vacuum, then blowing heated air through a continuous spray of the hot cream while still maintaining a partial vacuum until the cream is free from all removable off-flavors, as determined by drawing samples from the vacuum apparatus.

5. The time necessary for this treatment varies with type and intensity of flavor defect, it ranges from instantaneous treatment, in which the cream passes from the pasteurizer through the vacuum and cooler into the holding tank in one continuous operation, to intermittent or batch treatment in which the cream is held and treated under vacuum for from fifteen to thirty minutes and even longer in extreme cases.

6. In order to avoid all danger of premature churning and insure butter with a smooth texture free from mealiness, it is necessary to keep the cream in motion until cooled and to cool it rapidly and without excessive agitation.

7. While the application of this method of removing flavors from cream for buttermaking has been reduced to commercially successful and practical operation, some of the technical phenomena encountered in this work require further study for satisfactory explanation of the scientific principles involved. The most important of these are: The ratio of evaporation, the behavior of individual flavors, conditions controlling their volatilization, and the physical changes of the fat units. These problems are now under investigation and their solution promises the revelation of additional interesting and valuable scientific facts.

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# RELATION OF WATER TO MILK BORNE TYPHOID*

CARL R. FELLERS[1]
*University of Washington, Seattle, Washington*
AND
ROY S. DEARSTYNE
*North Carolina Agricultural and Mechanical College, Raleigh, North Carolina*

## I. LONGEVITY OF BACT. TYPHI AND ALLIED SPECIES OF BACTERIA ON DAIRY UTENSILS AND IN WATER

In the course of extensive field and laboratory work on dairy and milk inspection problems in the South, the authors became thoroughly convinced of the tremendous importance of a safe water supply on dairy farms. This is not by any means an original or new idea for it has been pointed out before by a number of able sanitarians and scientists. For example Health Officer Letter No. 13 of the Bureau of Animal Industry, United States Department of Agriculture, issued in 1918 deals entirely with this subject. Nevertheless there is a well recognized dearth of actual data based upon experimental work upon which the sanitarian or milk inspector may draw. This paper is presented therefore for the purpose of furnishing some definite information upon the following points: First, the longevity of certain bacteria of the typhoid group upon dairy utensils and in water; second, the extent of pollution of certain dairy farm water supplies and third, the efficiency of various methods of washing and sterilizing dairy utensils, particularly bottles.

A very brief résumé of the more important investigations on this subject is presented as a useful adjunct to the experimental data which follows.

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* Received for publication June 20, 1924.

[1] Joint contribution from the Department of Food Preservation, University of Washington, Seattle, and Pathological Laboratory, North Carolina Agricultural and Mechanical College, Raleigh, North Carolina.

As a result of investigating 638 epidemics of typhoid fever Schuder (8) concludes milk caused the disease 111 times or 17 per cent. Trask (27) investigated 179 typhoid outbreaks due chiefly to milk and found 113 of these were traceable to farms, dairies or milk shops. Four outbreaks were traced to infected milk bottles, two to infected milk utensils and others to a variety of causes.

Germano (4) concludes drying destroys typhoid bacilli but where there is a trace of moisture as in clothing, dirt or fecal filth, they may retain their vitality for a long time. A few resistant individuals resisted drying a very long time though most of the organisms were destroyed in a short time. Neisser (16) does not believe it possible to spread typhoid by dust though Flugge (5) claims bacilli may remain alive for 15 days. Robertson (22) found that typhoid bacilli may live in soil for at least several months whereas Martin (15) showed their longevity in soil varied according to the character of the soil flora. Viable cells of *Bact. typhi* were recovered from dried soil after 456 days. In fresh milk it is known that the typhoid bacillus may find a very favorable medium for growth. In this connection Washburn (28) found it to survive several months in fresh sweet milk. Northrup (17) demonstrated milk must become 80 degrees acid before *Bact. typhi* is destroyed thus partly substantiating Krumweide and Noble (14) who report it is killed in sour cream and acids and that the rate of destruction depends on both the acidity and the number of bacteria present. Bassenge (18) also states a 0.3 to 0.4 per cent acidity in milk will destroy typhoid bacilli after 24 hours. On the other hand Potter (25) asserts that lactic acid bacteria exert no repressing effect on *Bact. typhi* and will not render harmless a contaminated milk supply. Herzog (7) states this organism generally finds its way into milk through contaminated water used to wash the dairy utensils. He finds milk exerts first a depressing effect on the organisms followed by a marked increase in numbers.

Jordan, Russel and Zeit (12) reported that *Bact. typhi* died in 7 days in lake water and in about 3 days in polluted water. Later Russel and Fuller (26) working under somewhat different conditions, confirmed these results in a general way. Houston (9) determined that it required 9 weeks for the total disappearance of *Bact. typhi* from river water, though 99 per cent of the organisms died in one week. He further concluded that the rate of death of this organism in pure natural water follows the velocity law for monomolecular reactions. Phelps (20), also Cohen (2) have further verified this work. The latter investigator points out the importance of temperature and hydrogen ion concentration in this connection. The optimum pH value for *Bact. typhi* was shown to be from 5.0 to 6.4. Whipple (29) in reviewing longevity experiments states the results are contradictory, but considers few living cells will survive 25 days exposure in water. Salt, lake and well water are all declared to be unfavorable. Jordan (11) and also Gay (3) hold substantially the same views regarding the longevity of this organism. Park and Williams (19), Harrison (6), and Harrington and Richardson (5) are in accord in stating that the majority of cells of *Bact. typhi* are destroyed in 7 or 8 days in pure water, in a shorter time in very cold or in polluted waters, and that only a few resistant individuals survive after the first few days.

Sedgwick and Winslow (23) found freezing is very destructive to typhoid bacilli, over 90 per cent being destroyed in 2 weeks. A very few organisms were recovered after 12 weeks in ice. Prudden (21) demonstrated living cells in ice 103 days after infection. He states they may remain viable for several weeks in soil and in pure water. Bolten (1) determined the presence of living *Bact. typhi* in ice cream after a storage period of 45 days. According to Ishii (10) this organism dies out in glucose or mannite peptone media in from 3 to 8 days while in lactose or saccharose broth it survived for over a month. Young and Sherwood (30) also Koser and Skinner (13) investigated the effect of carbonated beverages on bacteria and agree in finding the numbers of *Bact. typhi* greatly reduced after a few hours contact, yet some few cells remained viable for several days.

## EXPERIMENTAL

*Preliminary experiment 1.* One loopful of a diluted twenty-four culture of *Bact. typhi* Hopkins strain (Hygienic Laboratory), was placed in each of a series of dry sterile petri dishes. Direct microscopic examination showed about 20,000 bacteria per loop. The loopfuls of saline dried in about 10 minutes. The storage chamber was dark and was maintained at a temperature of from 20° to 23°C. Plates were removed at the end of the following periods of time: 1, 2, 4, 6, 8, 12, 16, 24, 36 and 48 hours. Two plates were tested at each period by the addition of plain peptone, beef extract agar pH value 7.0, to one plate and Endo agar to the other. Plates were then incubated at 37°C. for as long as necessary to determine whether growth was present. Accurate numerical estimation of the colonies was not possible and for this reason the growth was classed as +, ++, +++, ++++ according as the growth was scant or vigorous. It was necessary to examine the plates several times as the appearance of the characteristic colonies of *Bact. typhi* was occasionally delayed for 4 or more days. The results of this preliminary experiment are incorporated in table 1. The most rapid diminution in numbers occurred from the second to the fourth hour. At the 48 hour testing period approximately 75 per cent of the organisms had been destroyed. No tests were made after 48 hours because all the test plates had been used.

*Experiment 2.* This is a repetition of experiment 1 with the exception that the drying period was much extended and several other species of bacteria were used. The detail of the experiment was identical in every way except duplicate plates of both plain and Endo agar were used at each test period. This was done to avoid discrepancies observed in the former experiment. The data are contained in table 1, only a brief discussion of the results being necessary.

*Bact. typhi* Hopkins strain, survived (2 out of 4 plates) 96 hours but not 120 hours. *Bact. typhi* United States Army strain survived 168 but not 192 hours. An average of only

TABLE 1

*Longevity of typhoid group when dried on glass plates or rods*

| TIME ELAPSED | EXPERIMENT 1 | EXPERIMENT 3 | EXPERIMENT 2 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Bact. typhi* on glass plates | *Bact. typhi* on glass rods | *Bact. typhi* Hopkins | *Bact. typhi* Army | *Bact. paratyphosum A* | *Bact. paratyphosum B* | *Bact. dysenteriae* Shiga | *Bact. dysenteriae* Flexner | *Bact. enteritidis* |
| *hours* | | | | | | | | | |
| 1 | ++++* | +++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| 2 | ++++ | +++ | ++++ | ++++ | +++ | ++++ | ++++ | ++++ | ++++ |
| 4 | +++ | +++ | ++++ | ++++ | +++ | ++++ | ++++ | ++++ | ++++ |
| 6 | +++ | +++ | ++++ | ++++ | ++ | +++ | ++ | ++ | ++++ |
| 8 | ++ | +++ | ++++ | +++ | + | ++ | ++ | ++ | ++++ |
| 12 | + | +++ | +++ | +++ | — | ++ | ++ | — | ++++ |
| 16 | + | No test | No test | No test | No test | No test | No test | No test | No test |
| 18 | No test | +++ | +++ | +++ | — | — | +(sl.) | — | +++ |
| 24 | + | ++ | ++ | +++ | — | — | — | — | +++ |
| 36 | + | ++ | +++ | ++ | — | — | — | — | +++ |
| 48 | + | + | + | ++ | — | — | — | — | ++ |
| 72 | No test | — | + | ++ | — | — | — | — | ++ |
| 96 | No test | — | +(sl.) | + | — | — | — | — | + |
| 120 | No test | — | — | + | — | — | — | — | + |
| 144 | No test | — | — | — | — | — | — | No test | + |
| 168 | No test | + | — | +(v. sl.) | — | — | No test | No test | + |
| 190 | No test | — | — | — | — | — | No test | No test | — |
| 214 | No test | — | — | — | — | — | No test | No test | — |
| 260 | No test | — | — | — | — | — | No test | No test | — |

*+ represents scanty growth or very few colonies.

++++ represents vigorous growth or a large number of colonies.

2 colonies per plate was obtained at the 168 hour testing period thus showing that all except a few resistant individuals had been destroyed long before this test period. *Bact. paratyphosum* A survived 8 but not 12 hours, most of the organisms being killed within 2 hours after being placed on the glass dishes. *Bact. paratyphosum* B withstood desiccation 12 but not 24 hours. The plates poured at the 18 hour period using the latter organism showed only 2 colonies on the 4 plates. It is evident that the life of the dysentery group of bacteria is limited to a few hours at most when dried under these conditions, because *Bact. shigae* survived 8 but not 12 hours and *Bact. dysenteriae* Flexner survived the 12 but not the 24 hour period. Most of the living cells of both of these species were destroyed during the first 4 hours of exposure. In the case of the latter only a total of 2 colonies were discovered on a total of 4 plates under examination at the 18 hour testing period. On the other hand *Bact. enteritidis* survived 168 hours of desiccation though the number of surviving organisms after the 96 hour test period was very small. No colonies were found after 192 hours in storage.

*Experiment 3.* In this experiment the bacteria were dried on sterile glass rods. A 24 hour culture, diluted with about 10 times its volume of water, was used as the inoculant. A direct microscopic count showed there were less than 100,000 organisms per rod. The rods were dipped to a depth of about ½ inch in the diluted culture, drained 30 seconds, touched to the side of the tube and transferred at once under aseptic conditions to large cotton-stoppered test tubes for storage. Upon examination it was found that the culture appeared perfectly dry in 4 hours. In order to make the test for the presence of living bacteria the glass rods were transferred to tubes of plain broth and incubated. All tests were made in triplicate, that is 3 glass tubes were used at each test period. The results are found tabulated in table 1. *Bact. typhi* Hopkins was the organism used.

It was found that all cultures made up to 24 hours were strongly positive. After this length of time had elapsed however, nearly all the organisms had been destroyed. For example one tube appeared sterile after 24 hours, two tubes after 36 hours and 2 tubes after 48 hours. In the latter case, the growth did not appear until after 5 days incubation, due no doubt to the impairment of the vitality of the organism. After the 48 hour test period no more positive cultures were obtained from the rods until the 168 hour test. From one of the tubes atypical culture of *Bact. typhi* was obtained. From these and other results obtained throughout this work it appears that occasionally very resistant individual cells are encountered. In every case these resistant types were true to the species type and although their pathogenicity was not tested, it seems probable that it remained unimpaired. A very great majority of the cells are readily destroyed after a very few hours drying.

*Experiment 4.* It was determined to test out the effect of a milk film upon the longevity of some of the typhoid group. For this purpose the loop method already described was used. First a drop of sterile milk was placed in a sterile petri dish and spread out well with the aid of a platinum wire. Then a loop of a diluted 24 hour culture of the organism being tested was spread about in the milk film in the glass dish. Check plates made at the same time and under identical conditions but containing no milk film were used. All results are reported in table 2. Tests were made in triplicate, that is 3 plates were used as checks and contained no milk, while 3 contained both culture and milk.

Upon consulting table 2 it is seen that *Bact. typhi* survived 115 hours when dried alone without milk on the petri dishes. One of the 3 plates showed a few colonies after the 140 hour test period but all other plates examined after 115 hours storage were negative. When dried in contact with the milk film, large numbers of colonies of this organism were present on every plate even after the final test, i.e., 250 hours. Just why this dried milk film should protect bacteria for such a long time is not entirely clear. It is probable that the moisture and protective colloids which are present are important factors. Similar results were obtained with *Bact. paratyphosum* A when dried with a milk film. In this case many colonies were found on the plates after the 192 hour test and a few (30 colonies) on the 3 plates tested at the 216 hour period. Where no film of milk was present the bacteria were all killed in 24 hours and only a few resisted 18 hours storage. *Bact. dysenteriae* without the milk film did not resist the 43 hour period, in fact nearly all the cells were destroyed after 6 hours. One doubtful plate showed 3 colonies of after the 67 hour test but all other tests made after the 43 hour period were negative. Where milk was present the organisms survived 140 hours desiccation but not 168 hours. Large numbers of colonies were found on the plates made at the 115 and 140 hour periods.

TABLE 2

*Effect of milk films on the longevity of typhoid group when dried on glass plates*

Experiment 4

| TIME ELAPSED | BACT. TYPHI ALONE | BACT. TYPHI AND MILK FILM | BACT. PARATYPHOSUM A ALONE | BACT. PARATYPHOSUM A AND MILK FILM | BACT. DYSENTERIAE FLEXNER ALONE | BACT. DYSENTERIAE FLEXNER AND MILK FILM |
|---|---|---|---|---|---|---|
| *hours* | | | | | | |
| 1 | +++ | +++ | +++ | +++ | +++ | +++ |
| 2 | +++ | +++ | +++ | +++ | +++ | +++ |
| 4 | +++ | +++ | +++ | +++ | ++ | +++ |
| 6 | +++ | +++ | +++ | +++ | ++ | +++ |
| 8 | +++ | +++ | + | +++ | ++ | +++ |
| 12 | +++ | +++ | + | +++ | ++ | +++ |
| 18 | +++ | +++ | + | +++ | ++ | +++ |
| 24 | +++ | +++ | − | +++ | + | +++ |
| 36 | ++ | +++ | − | +++ | − | +++ |
| 43 | ++ | +++ | − | +++ | − | ++ |
| 67 | + | +++ | − | ++ | + | ++ |
| 96 | + | +++ | − | ++ | − | ++ |
| 115 | + | +++ | − | ++ | − | + |
| 140 | + (sl.) | +++ | − | ++ | − | + |
| 168 | − | +++ | − | +++ | − | − |
| 192 | − | +++ | − | ++ | − | − |
| 216 | − | +++ | − | + | − | − |
| 250 | − | +++ | − | − | − | − |

### *Discussion of the desiccation experiments*

These experiments indicate that *Bact. typhi* and to a lesser degree *Bact. paratyphosum* A and *Bact. dysenteriae,* in spite of the fact that they are non-spore formers, are able to withstand considerable desiccation before being destroyed. In thin films on glass plates or rods *Bact. typhi* (2 strains) showed survival after 92 hours and in one case even after 168 hours. Where a thin film of milk was present in addition to the bacterial film, the organisms from 7 to more than 10 days. Similar results were obtained with the dysentery and paratyphoid cultures, though neither appears to be as resistant as the typhoid bacillus. In the case of the 2 strains of the latter organism tested, they were still viable after from 5 to 7 days when dried on glass with a thin film of milk.

These data show clearly the necessity of using pure water to wash milk bottles and other utensils, for if any of the intestinal bacteria were present they could easily survive long enough to infect the milk. Often the length of time elapsing between the time of washing utensils at the dairy and the time of the next milking is only a few hours. In spite of laws to the contrary in many cities and towns, the rinsing of dairy utensils after the usual superficial disinfection, is sometimes done. Not only is this washing and rinsing often done with polluted water but the entire cleansing process often consists of a superficial washing of the bottles and utensils in luke-warm water with a little soap or cleaning preparation. The removal of all traces of curdy and slimy particles from milk bottles and utensils is also of the greatest importance because bacteria of the typhoid-dysentery type are able to survive in thin films of milk for a week or 10 days without apparent loss of vitality. It is also well known that steam or a disinfecting solution do not readily penetrate beneath such curd or slime areas. It is believed that thorough cleansing of all utensils, in addition to the use of a good steam cabinet or an efficient liquid disinfectant, is essential in protecting the public from the dangers of typhoid infection from polluted water or carriers. Of course wherever pasteurization is effectively done, this serves as an additional safeguard against the spread of infection from milk.

Steam sterilization for the disinfection of bottles and other utensils is used to good advantage in most of the large well equipped milk plants but the small dairyman must have recourse to either an easily prepared disinfectant which must be both harmless yet efficient, or else a simple steam sterilization outfit. Several such disinfectants including chlorinated lime and similar substances have been suggested. Some data obtained regarding the efficiency of various methods of cleaning and sterilizing milk utensils, particularly bottles will be included in part III of this paper.

### *Longevity of typhoid group of bacteria in surface, mineral and sea water*

For these experiments large, hard, white glass reagent bottles containing 7 liters were used. The organisms tested were *Bact. typhi* Hopkins strain, *Bact. paratyphosum* A and *Bact. shigae*. The bottles containing 5 liters of water each were sterilized by heating in flowing steam for one hour, inoculated when cool with a 24 hour culture (1:10) of the organism to be tested and stored in a very subdued light at a mean temperature of 60.9°C. Samples for examination were removed by means of sterile pipettes. The tap water used was unchlorinated Seattle tap water from Cedar River, the salt water from Puget Sound near Seattle while the mineral water was artificially prepared in the laboratory to closely simulate White Rock Lithia Water. Three media were used in making the tests. These were plain broth, plain agar (A. P. H. A.) and Endo agar. The purpose of the peptone broth was to serve as a check on the solid media. At each testing period colonies were fished from the plates and occasionally cultured to make certain of their identity. No contaminations occurred until after the 3 months testing period when white and yellow micrococci were found in 2 of the bottles. One of these was inoculated with *Bact. paratyphosum* A and the other with *Bact. shigae*, both in mineral water. The results in full are incorporated in table 3.

TABLE 3

*Longevity of typhoid group in surface, salt and mineral waters*

| TIME OF STORAGE | BACT. TYPHI HOPKINS ORGANISMS PER CUBIC CENTIMETER | | | BACT. PARATYPHOSUM A ORGANISMS PER CUBIC CENTIMETER | | | BACT. DYSENTERIAE SHIGA ORGANISMS PER CUBIC CENTIMETER | | |
|---|---|---|---|---|---|---|---|---|---|
| | Tap | Sea | Mineral | Tap | Sea | Mineral | Tap | Sea | Mineral |
| 2 hours | 1,300 | 1,920 | 1,140 | 1,100 | 1,160 | 1,130 | 1,700 | 2,530 | 3,600 |
| 22 hours | 1,530 | 6,100 | 7,200 | 820 | 3,180 | 5,640 | 1,080 | 2,100 | 8,000 |
| 46 hours | 580 | 25,000 | 35,000 | 1,730 | 20,000 | 50,000 | 800 | 2,280 | 50,000 |
| 72 hours | 300 | 50,000 | 80,000 | 1,400 | 20,000 | 50,000 | 450 | 50,000 | 1,050 |
| 96 hours | 240 | 80,000 | 100,000 | 1,140 | 50,000 | 80,000 | 200 | 80,000 | 900 |
| 144 hours | 40 | 100,000 | 100,000 | 830 | 170,000 | 170,000 | 30 | 170 | 100,000 |
| 7 days | 10 | 350,000 | 700,000 | 100 | 1,800,000 | 1,600,000 | 16 | 100 | 600,000 |
| 9 days | 6 | 300,000 | 800,000 | 100 | 1,600,000 | 800,000 | 1 | 20 | 800,000 |
| 11 days | 6 | No test | No test | 150 | No test | No test | 0 | 30 | No test |
| 13 days | 8 | 150,000 | 300,000 | 10 | 720,000 | 265,000 | 2 | 6 | 480,000 |
| 15 days | 3 | 120,000 | 150,000 | 0 | 600,000 | 200,000 | 0 | 0 | 1,000,000 |
| 21 days | 0 | 70,000 | 80,000 | 0 | 240,000 | 120,000 | 0 | 0 | 100,000 |
| 30 days | 0 | 30,000 | 25,000 | 0 | 10,000 | 400,000 | 0 | 0 | 60,000 |
| 3 months | 0 | 5,000 | 20,000 | 0 | 20,000 | 80,000 | 0 | 0 | 20,000 |
| 6 months | 0 | 200 | Positive | 0 | 600 | Positive | 0 | 0 | Positive |

### *Discussion*

Considering first of all the action of tap or surface water upon these organisms, it was found that all 3 species tested were destroyed between the thirteenth and twenty-first days. *Bact. typhi* survived longest followed closely by *Bact. paratyphosum* A and *Bact. shigae.* The death rate follows closely the logarithmic curve of the velocity law as related to a monomolecular reaction. A few resistant individual cells linger for many days before finally succumbing, though a great majority are destroyed in the first 7 days.

In salt water the death rate was much different from that found in surface water, and was different for each species tested. Considerable multiplication took place in all species used though *Bact. shigae* did not multiply to the same extent nor live as long as the other bacteria. Some living cells of both *Bact. paratyphosum* A and *Bact. typhi* were recovered from the samples after 6 months storage. All 3 species showed a gradual decrease followed by a similar gradual increase in numbers in salt water. Although this water was not analyzed it is possible that traces of organic matter sufficient for the needs of these organisms was present. It is apparent from this experiment at least that sea water like normal saline forms a more favorable medium to resist destruction than relatively pure surface water.

The results obtained with these 3 organisms in mineral water are in general similar to those just described where salt water was used. However the organisms multiplied to a much greater extent in the mineral water and all were still in a viable condition at the end of 6 months when the experiment was concluded. No organic matter was intentionally added; the large increase in the numbers of organisms cannot therefore be ascribed to the presence of more than a trace of organic matter. One-tenth cubic centimeter of a 1:10 peptone broth was added to the water as the inoculant. The amount of organic matter transferred in this matter was very small indeed. At any rate the data substantiate results obtained by other investigators respecting the longevity of these bacteria in certain kinds of water. One reason why results in the literature are so discordant is undoubtedly because of the great difference in the character of the water samples employed as well as other experimental conditions which are difficult to duplicate in different laboratories.

### *Effect of direct sunlight on the viability of the typhoid group in water*

This experiment was an exact duplicate of the former one except that tap water only was used, and the bottles were stored in a window exposed to the direct rays of a June sun during the greater part of the day. The results are given in table 4.

Table 4 shows that the 2 strains of *Bact. typhi* and the one strain of *Bact. typhosum* A tested in this experiment had completely died out in 4 days, probably before, but due to unforseen circumstances tests could not be made at the end of 48 and 72 hours as originally planned. Again *Bact. typhi* appears to be more resistant than *Bact. paratyphosum* A, for only 1 per cent of the latter survived even 24 hours while the former did not greatly diminish in numbers until after the first 24 hours. The action of direct sunlight upon these bacteria in tap water is probably one of the principal causes for the speedy destruction of the living cells, at least other conditions were made identical to the previous experiment where the organisms survived for a much longer time when not exposed to direct sunlight.

TABLE 4

*Effect of sunlight upon the viability of typhoid group in surface (tap) water*

| DURATION OF STORAGE | BACT. TYPHI HOPKINS ORGANISMS PER CUBIC CENTIMETER | BACT. PARATYPHOSUM A ORGANISMS PER CUBIC CENTIMETER | BACT. TYPHI ARMY ORGANISMS PER CUBIC CENTIMETER |
|---|---|---|---|
| *hours* | | | |
| 1 | 15,000 | 10,000 | 12,000 |
| 24 | 9,500 | 100 | 12,000 |
| 96 | 0 (1 cc.) | 0 (1 cc.) | 0 (1 cc.) |
| 120 | 0 (2 cc.) | 0 (2 cc.) | 0 (2 cc.) |

The practical application of these longevity experiments in water is obvious. The relation of them to farm and public water supplies will not be discussed here because it is our motive to point out the danger of an unsafe water supply on dairy farms to the milk consuming public. The water may be used to wash or rinse utensils and bottles and thus transfer the harmful organisms directly to the milk. Then of course is the danger of carriers who have contracted the disease by consuming the infected water. It has been demonstrated by the desiccation experiments that typhoid and allied bacteria may live many hours on glass utensils, and even a week or more when a thin film of milk protects them. It has also been shown that these organisms are able to retain their vitality in surface, mineral and salt water for at least several days thereby increasing the chances of infecting the milk supply. It will be seen therefore that the danger of a polluted farm dairy water supply is indeed a real one. Data bearing on the pollution of these wells is presented in Part II of this paper.

## *Summary*

*Bact. typhi*, *Bact. enteritidis* and to a lesser extent *Bact. paratyphosum* A and the dysentery bacteria are able to withstand desiccation upon glass for periods of time varying from 8 hours to several days. In general *Bact. typhi* was the most resistant as in 2 cases it resisted 96 hours and in one case even 168 hours. The longevity of this group of bacteria varied considerably in the different tests.

Where a thin film of milk was present with the dried culture, the above organisms survived for from 7 to 10 days, thus greatly increasing the time necessary to destroy them. The film of milk acts as a protective coating and through the retention of moisture or other reasons effectively preserves the vitality of the bacteria for several days.

The rate of destruction in every case was greatest during the first few hours of drying. A few resistant individual cells still survived long after the great majority of the organisms had been destroyed.

*Bact. typhi*, *Bact. paratyphosum* A, and *Bact. shigae* when placed in a pure surface water survived from 13 to 20 days.

The death rate followed closely the logarithmic velocity curve of a monomolecular reaction. In salt and in mineral waters these organism decreased for a time then greatly increased in numbers so that, except for *Bact. shigae* in salt water, all survived in small numbers even after 6 months storage in the dark. It is believed that a sufficient amount of organic matter for limited bacterial growth was present in these water samples in order to account for such a long survival.

Using surface water alone stored in the direct sunlight, it was found that *Bact. typhi* and *Bact. paratyphosum* A were destroyed in less than 4 days.

The data indicate that bacteria of the typhoid group are able to survive in water and on glass surfaces for a sufficient length of time to infect milk on the average farm. All milk utensils should be washed with great care as it was demonstrated that a thin milk film or curd particles protect bacteria from destruction by desiccation. There is a real danger in using polluted water for the purpose of washing or rinsing dairy utensils. Since many wells and springs on dairy farms are polluted, an efficient but non-poisonous disinfectant such as chloride of lime or hypochlorite should be used to sterilize utensils where steam is not available.

## II. EXTENT OF WATER POLLUTION ON CERTAIN DAIRY FARMS

Sanitary surveys supplemented by bacteriological examinations of dairy farm wells and other water supplies were conducted by the writers in Mecklenburg, Cabarrus and Gaston Counties, North Carolina. In carrying out this survey all pertinent epidemiological data such as typhoid history, type of well or privy, dairy score, etc., were carefully collected. A series of 50 dairy farm wells and springs were taken at random in this typical southern area and samples were carefully collected and examined. Each well was examined at least twice if it showed a positive colon test the first time. In case the first and second examinations did not check, a third examination was always made. The total count of bacteria on Standard Agar (American Public Health Association) at 37°C. for 48 hours as well as the presumptive and completed tests for the *Bact. coli* group, and differentiation of the latter into the fecal and non-fecal types, were made on every sample.

Table 5 gives a highly condensed summary of some of the epidemiological and bacteriological data collected regarding these dairy farm water supplies. In a large measure these data are self explanatory. For example it is evident at a glance that the open bucket and poorly protected types of wells showed the highest degree of contamination with the colon group. According to the present standards of the American Public Health Association (32), and water sample showing the presence of the *Bact. coli* group of the fecal or even the non-fecal types is classed as at least potentially dangerous. The 6 deep, drilled wells with pumps furnished water of exceptional sanitary quality—an excellent argument for the drilled well. A total of 38 wells or springs or 76 per cent showed the presence of the *Bact. coli* group of the fecal or non-fecal types; however, on differentiating these it was found that only 21 or 42 per cent actually contained fecal types. Fifteen samples contained both fecal and non-fecal types, while 7 contained the fecal type alone. Thus we see that the non-fecal members of the colon group are more than twice as numerous in these farm wells as are the

TABLE 5

*Bacteriologic and epidemiologic data on 50 representative farm dairy water supplies*

| TYPE OF SUPPLY | NUMBER | AVERAGE DEPTH | NUMBER PROTECTED FROM SURFACE DRAINAGE | AVERAGE DISTANCE NEAREST BUILDING | TOTAL COUNT BACTERIA PER CUBIC CENTIMETER | POSITIVE PRESUMPTIVE TEST FOR BACT. COLI GROUP | POSITIVE COMPLETED TEST FOR BACT. COLI GROUP | NUMBER SHOWING PRESENCE OF FECAL TYPE OF BACT. COLI GROUP |
|---|---|---|---|---|---|---|---|---|
| | | *feet* | | | | | | |
| Open bucket wells | 21 | 31.5 | 11 | 30.5 | 130 | 19 | 19 | 12 |
| Dug wells with pump | 17 | 36.8 | 12 | 38.0 | 61 | 14 | 13 | 6 |
| Flowing springs | 6 | Shallow | 3 | 275 | 33 | 6 | 6 | 3 |
| Drilled wells with pump | 6 | 75.5 | 6 | 23 | 5 | 0 | 0 | 0 |

fecal types. This is an important point and one which the authors feel has not been sufficiently dwelt upon in the past. It is a well known fact that no differentiations are made as to type in routine water examinations in most laboratories. Our results tend to corroborate Chen and Rettger (31) who found only 20 or 4.3 per cent of 467 cultures or colon-like soil organisms were actually fecal *Bact. coli*. Of the remaining 447 cultures, 430 were *Bact. aerogenes* and 17 were *Bact. cloacae.*

As stated before in this paper, there is a greater chance for dairy farm wells to become polluted with fecal colon bacteria than similar wells not located on dairy farms. This is because of the greater opportunity of cow dung getting into the water supply where cattle are kept. For example the authors in collaboration with Mr. L. R. Jones, bacteriologist, United States Public Health Service, examined one hunderd wells in the 5 mile extra-cantonment zone at Camp Greene, Charlotte, N. C., in 1918 and found 40 per cent were polluted by the undifferentiated colon test. Each well was examined at least twice. Since probably one-half of the wells classed as polluted contained non-fecal colon-like organisms only, it is evident that only about 20 per cent of the wells actually contained fecal *Bact. coli*. This percentage is only one-half that found in dairy farm water supplies from this same district.

In the first series of 50 wells, eight families gave positive typhoid histories, while one active case was found. Since active or carrier cases of this disease may readily infect bucket type or poorly protected wells or springs, and because milk utensils may be washed or rinsed in this same water, the danger from typhoid on these farms is indeed very real. Such danger is not present to the same extent in communities where pasteurization is practised, nevertheless we should go to the source of the trouble first of all. The water supply should be carefully safeguarded from pollution, in addition to this all utensils should be thoroughly washed and sterilized either by steam or some suitable disinfectant, and lastly as a final safeguard, the milk should be efficiently pasteurized.

### *Summary*

In a series of 50 dairy farm wells and springs tested bacteriologically, 38 or 76 per cent contained members of the *Bact. coli* group. In this series only 21 or 42 per cent actually contained fecal types of this group as differentiated by the methods of the American Public Health Association. Of 100 wells and springs in the same district but not from dairy farms 40 were found to be polluted by the undifferentiated colon test.

Dairy farm water supplies are more likely to be polluted with fecal bacteria than similar supplies on other farms because of the presence of cow dung.

According to some of the present methods of interpreting the results of a sanitary water examination, many samples are classed as polluted which really contain no fecal organisms.

## III. EFFICIENCY OF VARIOUS METHODS OF WASHING AND STERILIZING DAIRY UTENSILS

In cities and towns where the milk supply is marketed raw, the milk and dairy inspection department of these municipalities must always be on the alert for milk-borne epidemics. Few, if any epidemics of contagious disease have been traced to milk which has been properly pasteurized, because the heating and holding process of pasteurization (142° to 148°F. for 20 to 30 minutes) destroys non-sporeforming disease-producing organisms which might have found their way into the milk. Aside from this, the facilities for bottle and can sterilization in modern pasteurizing plants are usually such as to preclude chances of contamination of milk subsequent to the pasteurizing operation.

The means of entrance of disease producing organisms into the milk supply are many, but the object of this paper is primarily to correlate polluted water supplies with some of the milk borne diseases.

Nissen (36) found *Bact. typhi* cultures are killed in 10 minutes in a dilution of 0.06 per cent of chlorinated lime and in 5 minutes in a 0.12 per cent solution of this substance. This work was substantiated by Taylor (37) who asserts a chlorinated lime solution of such strength that there is one part available chlorine to 5000 parts of water, is very effective and completely sterilizes dairy utensils in a one minute exposure, providing the bottles and milk cans have been throughly scrubbed to remove particles of curd. Van Saun, Bancroft and O'Gorman (38), and also Berry (33) made a study of the glass and dish washing problem in restaurants, soda fountains and other public places. They conclude that strong chloride of lime solutions effect practically instantaneous sterilization, but that the disinfectant should be rinsed from the utensil. These investigators as well as Cole (34) and Manheimer and Ybanez (35) proved that unless a chemical disinfectant is used, boiling water is necessary to effect sterilization. Theoretically bottles and utensils should be efficiently sterilized and properly stored. If the sterilization has been adequate and the storage conditions suitable, the chances of infection from such sources as worked out in parts I and II of this paper are negligible, but, in cities in which milk regulation work is in its infancy, and which derive all or a part of their supply from small dairies marketing a raw product, the usual tendency of the dairyman is to adhere to the old time principles of sanitation and cleanliness. Most of these small dairies operate on a very narrow margin, being for the most part conducted in conjunction with diversified farming. There is often a shortage of bottles, cans and other utensils. Cleaning and sterilizing is rushed through at the last moment, the bottles perhaps superficially washed in soap solution, and dipped in so-called boiling water which is not boiling at all, but merely luke warm, rinsed and drained. Then too on the part of some of the dairymen, there is the inevitable tendency to clean out the cans and pails superficially, and then to sterilize and rinse them hurriedly. Despite the most rigid of laws and efficient systems of inspections, this practice is more or less in vogue at small dairies that lack live steam cabinets having capacity for sterilizing and storage. Hence we have polluted water a potent factor in the spread of milk-borne diseases.

The writers collected so-called sterile bottles ready for filling, under conditions as listed from several raw milk dairies, capped and carried them to the laboratory. Here 100 cc. of sterile water was placed in each, the bottle again capped and thoroughly agitated, proper dilutions made, plated and counted according to the standard methods of milk analysis of the American Public Health Association. The results undoubtedly represent a

TABLE 6

*Bacterial counts per bottle*

Bacteria per bottle

| WASHED IN HOT WATER AND RINSED WITH A COMMERCIAL HYPOCHLORITE DISINFECTANT* | | | WASHED IN HOT WATER | | LIVE STEAM | |
|---|---|---|---|---|---|---|
| Quarts | Pints | | Quarts | Pints | Quarts | Pints |
| 3,600 | 300 | 100 | 900,000 | 60,000 | Sterile | Sterile |
| 500 | 200 | Sterile | | | 200 | Sterile |
| 300 | 200 | 200 | 50,000 | 800,000 | 400 | Sterile |
| 1,500 | 100 | 500 | 100,000 | 660,000 | 500 | Sterile |
| 200 | 100 | Sterile | 120,000 | 1,500 | 500 | 200 |
| 200 | 400 | 500 | 1,000,000 | 300,000 | 400 | 100 |
| 800 | 100 | 100 | 600,000 | 8,000 | 200 | 100 |
| 200 | 100 | Sterile | 1,000,000 | 60,000 | 400 | 300 |
| 100 | 500 | 500 | 800,000 | 500,000 | 400 | Sterile |
| 200 | 2,000 | 200 | 63,000 | 50,000 | 900 | 400 |
| 500 | 500 | 300 | 10,000 | 2,000 | 1,200 | 100 |
| 200 | | | 3,400 | 5,000 | 200 | 25,000 |
| | | | 1,500 | 63,000 | 100 | 300 |
| | | | | 3,000 | 20,000 | 120 |
| | | | | 1,000 | 400 | Sterile |
| | | | | 20,000 | 800 | 200 |
| | | | | 4,000 | 100 | Sterile |
| | | | | 8,000 | | 300 |
| | | | | 800 | | |
| 692<br>Average for 12 quarts | 313<br>Average for 22 pints | | 387,000<br>Average for 12 quarts | 41,400<br>Average for 18 pints | 1,480<br>Average 18 quarts omitting 20,000 count. average is 395. | 1,430<br>Average for 19 pints. 120 average for 18 pints omitting a 25,000 count. |

*Containing about 0.02 per cent available Cl.

minimum count of the bacterial flora of the bottles, and while the count is practically inconsequential from the standpoint of grading milk on the numerical basis, it is highly dangerous owing to the questionable nature of the organisms which the bottle is adding to the milk. Table 6 contains the results of this experiment.

There is little question in the authors' minds that the bottle and utensil question is of paramount importance especially where the total milk supply, or part of it is marketed raw. In the smaller cities and towns where no pasteurization is carried on and little attempt is made to regulate the milk supply, the matter of polluted water on dairy farms is of paramount importance.

These data show that sodium hypochlorite was very useful even when used merely for rinsing, in keeping the numbers of bacteria per bottle to a minimum. Live steam was also efficient, but the curd must previously be well removed by washing else a high count will occasionally result. So called "hot water" washing is inefficient and unsatisfactory from a bacteriological standpoint.

The authors believe that the use of sodium hypochlorite is the equal or superior of steam in the sterilization of milk bottles and dairy utensils, and since it is relatively inexpensive and easy to use and entirely harmless, they strongly recommend its use.[2]

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# A COLOR TEST AND OTHER METHODS FOR QUALITY OF MILK[1]

TRANSLATED FROM THE FRENCH BY

B. H. NISSEN

*Research Laboratory, Blue Valley Creamery Company, Chicago, Illinois*

For the rapid testing of milk for cheese making or other special purposes where unsuitable milk may be received, three rapid methods are at hand:

1. Determination of the refractive index of the calcium chloride serum of the milk.
2. Determination of the amount of catalase.
3. Alcoholic-alizarin test. The latter gives the most useful information.

Ackerman's calcium chloride serum method is best known. This is briefly as follows: 30 cc. of milk are introduced into a cylinder 230 mm. long by 20 mm. diameter, 0.25 cc. $CaCl_2$ solution is added and the whole shaken vigorously. Then place a refrigerant tube 260 mm. long by 10 mm. wide in the cylinder. A holder containing 12 to 24 such cylinders is placed in a bath of rapidly boiling water, for fifteen minutes; the water condensation collects on the refrigerant tube and is returned into serum. The refractive index is then made of the serum at 17.5°C. The calcium chloride solution used above contains 200 grams made up to a liter with water. Specific gravity 1.1375 at 15°C. Diluted to 1:10 this gives a refractive index of 26.0 ($n = 1.33743$).

The catalase number or amount is determined by aid of the apparatus of Koestler which is the simplest of the many described for the purpose. This is a cylinder of glass graduated at 10 and 15 cc., and provided with a one hole rubber stopper, carrying a tube connected with a eudiometer tube of 12 cc. capacity with

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[1] The Rapid Analysis of Abnormal Milk by the Use of the Refractometer, by the Measurement of Catalase and by the Alcoholic-Alizarin Test. By Valencien and Panchaud. Le Lait, iii, 529 (1923).

0.1 cc. divisions. Ten cubic centimeters of milk and 5 cc. of oxygenated water containing 1 per cent $H_2O_2$ (about three volumes) are added, then whole shaken and stoppered. A combination of 12 such tubes is arranged in water at 25°, just covering the top of the eudiometer connection tubes. They are left immersed for about one minute to let the imprisoned air expand. The eudiometer tube filled with water is then inverted over the opening. After two hours time the amount of oxygen liberated by the catalase is read off on the eudiometer tube and reported on a basis of 100 cc. of milk.

The alcoholic-alizarin test of Morres consists in observing the color and modifications which 3 cc. of milk undergo when shaken with 3 cc. of neutral alcohol of 68 per cent strength saturated with alizarin (about 2 grams per liter) which acts as an indicator. For pure lactic acid fermentation of about 8° Soxhlet-Henkel (S.H.) or 18° Dornic (D.) (see table of colors for definition of these) the mixture of milk and alcohol-alizarin does not coagulate but takes a lilac-rose color. At 9° S.H. (20° D.) the liquid is sprinkled with fine flakes, while at 16° S.H. the liquid is very much coagulated and the serum separates. The color is lemon yellow. As one enters this limit of acid a very marked change in color is noted, the more so as the acidity is increased. The coagulation is also more marked and the flakes larger.

But under the influence of bacteria secreting the ferment "rennet," purely a chyme fermentation results, with no increase in acidity. It is observed, however, that the alcohol-alizarin milk mixture is much coagulated and even if the acidity is less than 8° S.H., the color is dark brick red. Milk from a diseased udder shows a violet color indicating alkalinity, since alizarin is yellow in acid and violet in alkaline solution. This is especially unfit for cheese. In warm weather or in the shipping of milk the test is applied by taking an equal quantity of milk and alcoholic-alizarin. All milk giving a yellow color is set aside to be used in cheese or butter making. In this way only good milk is put into refrigeration cans.

Tillman has determined the H-ion concentration of fresh milk to which lactic acid was added to give the alcohol-alizarin colors

1 to 10 by the chart of Morres. (The same colors are here obtained as with the natural fermentation.) The H-ion concentration expressed in pH for these colors is as follows:

1 = 6.53
2 = 6.49
3 = 6.10
4 = 5.83
5 = 5.17
6 = 4.57
7 = 4.06
8 = 3.70

By addition of sodium carbonate alkaline milk is formed giving the following pH:

9 = 6.61
10 = 6.83

By the aid of such colors one can therefore obtain an approximate H-ion concentration of any sample of milk.

The refractive index of the $CaCl_2$ serum of normal milk from a healthy cow varies from 38.5° to 40.5° Zeiss ($n = 1.34218$ to $n = 1.34294$). A very low index results either from "watering" or from trouble in the secretion of the mammary glands. Fresh normal milk has a catalase number varying from 3 to 15 and if the milk is secreted by diseased cattle the catalase value is much higher, from 20 to 130 according to the degree of secretion trouble. On the other hand, such diseased milk shows a violet color with alcoholic-alizarin. Thus the accumulation of the three characteristics, very low refractive index, elevated catalase number and violet color with alcoholic-alizarin is very good proof that the milk is from diseased cattle.

The catalase value generally runs parallel to the leucocyte index of Trommsdorf, a high catalase value corresponds to an abnormal functioning of the mammary gland. Presence of blood, however, raises the amount of catalse, even if present in traces. By the examination of the centrifuged precipitate blood can easily be distinguished from leucocytes. We have even examined a milk which had a high catalase value, often very low instead.

To illustrate the rapidity by which one may draw conclusions from these preliminaries, we will mention the case of a cheese maker in whose manufacture we had noticed considerable trouble in 1913. One morning at eight o'clock we took 40 samples of milk obtained from a territory about 15 kilometers from Geneva. It was necessary that the cheese maker know by eleven o'clock what milk was to be suspected so it could be saved out from the rest. At the appointed hour this information was furnished him. The detailed condition of these samples of milk is given in tabular form showing the relation between the refractive index, the catalase value, and the alcohol-alizarin test.

(Four short tables were included in the original showing the relation between refractive index, catalase value, and alcohol-alizarin color for various samples of milk. A summary of these tables is given herewith.)

| NUMBER OF SAMPLE | REFRACTIVE INDEX | CATALASE VALUE | ALCOHOLIC-ALIZARIN COLOR |
|---|---|---|---|
| V 14-1 | 38.6 | 8 | Rose |
| V 14-2 | 37.0 | 43 | Lilac rose |
| A 2-2 | 39.1 | 12 | Rose lilac |
| A 2-4 | 39.1 | 10 | Rose lilac |
| A 2-5 | 38.9 | 21 | Rose lilac |
| A 2-7 | 39.3 | 50 | Rose (coagulated) |
| A 2-10 | 38.5 | 22 | Violet (abnormal) |
| V 1-1 | 39.0 | 13 | Rose lilac |
| V 17-9 | 39.3 | 18 | Rose lilac |
| V 14-2 | 37.8 | 20 | Violet (abnormal) |
| A 2-9 | 39.0 | 25 | Violet (abnormal) |
| A 9-1 | 40.1 | 12 | Red brick (with deposit, abnormal) |
| B 20-1 | 38.3 | 21 | Violet (slight deposit, abnormal) |

These samples show that it is very easy to tell what milk is normal and what is abnormal.

From the above it would appear that the most reliable information is given by the alcoholic-alizarin method.

*Translator's note:* The rapid colorimetric method discussed in the above and given more in detail in the following should be of especial interest to dealers of milk and others desiring a simple method for acidity: Short, 2 cc. milk pipettes, 5-inch test tubes, and a small bottle of the alcohol-alizarin solution constitute the necessary materials. Seventy-two parts of 95 per cent alcohol, 28 parts of water, and 1 part of a 20 per cent alizarin paste (alizarin is sold in a paste of this strength), mixed and filtered after standing. This with a few drops of N/10 NaOH produces the red normal, alizarin color. It was found to be fairly easy to distinguish milk differing by actual titration as little as 0.01 or 0.02 per cent so that if the alizarin indicator is of the right color (normal) to begin with, no difficulty should be experienced in obtaining results agreeing well with the usual titration method.

The following table gives the colors obtained with an alcohol-alizarin solution by which the nature of the change and the degree of acidity of milk can be approximated.

"S" indicates acidity in degrees Soxhlet, the number of cubic centimeters of N/4 NaOH required to neutralize the acidity of 100 cc. of milk.
"D" indicates acidity in degrees Dornic, the number of cubic centimeters of a NaOH solution containing 4.445 grams NaOH per liter required to neutralize the acidity of 100 cc. of milk.
1° Soxhlet or Soxhlet-Henkel = 2.25° Dornic (milk kept at 20°C.).

Degrees Dornic can be considered as acidity in hundreds per cent. Thus for a color of Rose (2°) the acidity would be approximately 0.18 per cent figured as lactic acid.

*Preparation of the solution and method of using.* Neutral alcohol is diluted with water to 68 per cent, 1.5 to 2 grams powdered alizarin, or the equivalent in paste form, is introduced into a liter of the above alcohol. This is allowed to stand and is filtered after six to twelve hours. If the filtrate is not of the desired color (aged bordeaux) it is adjusted to the right color (normal tint) by the addition of N/10 NaOH or N/10 acid.

To use, mix 2 or 3 cc. of milk with an equal quantity of the alizarin solution in a tall cylinder and observe the color and degree of coagulation. Compare with the scale of colors 1° to 10° given on the chart as follows:

<table>
<tr><th>NORMAL COLOR (WINE RED)</th><th>COLOR OF ALCOHOL-ALIZARIN</th></tr>
<tr><td>1° Red lilac</td><td>7° S. = 16° D. (approx.)</td></tr>
<tr><td colspan="2">Fresh normal milk. No precipitation. No coagulation after 7 hours boiling. No spontaneous coagulation after 12 hours</td></tr>
<tr><td>2° Rose</td><td>8° S. = 18° D. (approx.)</td></tr>
<tr><td colspan="2">First stage of acid formation. No precipitation; at most very fine flakes. Coagulates spontaneously after 9½ to 12 hours. Resists boiling 5 to 7 hours</td></tr>
</table>

<table>
<tr><th>NORMAL COLOR (WINE RED)</th><th>COLOR OF ALCOHOL-ALIZARIN</th></tr>
<tr><td>3° Red brown</td><td>9° S. = 20° D.</td></tr>
<tr><td colspan="2">Acidity has developed. Precipitation in fine or very fine flakes. Resists boiling for 3½ to 5 hours. Coagulates spontaneously after 7½ to 9 hours. If the color is darker and the precipitate more abundant and coarser, mixed lactic and cheese fermentation is indicated. Merchandising value questionable</td></tr>
<tr><td>4° Brown red</td><td>10° S. = 22°D.</td></tr>
<tr><td colspan="2">Acid formation continued. Fine flaky precipitate. Resists boiling 1½ to 3 hours. Coagulates spontaneously after 6 to 7½ hours. If the color is darker and the precipitate coarser or more abundant, indicates mixed fermentation</td></tr>
<tr><td>5° Brown</td><td>11° S. = 25° D.</td></tr>
<tr><td colspan="2">Critical state. Precipitate in flakes large and small. Resists boiling ½ to 1 hour. Coagulates spontaneously after 4½ to 6 hours</td></tr>
<tr><td>6° Brown yellow</td><td>12° S. = 27°D.</td></tr>
<tr><td colspan="2">The milk has reached the limit of resistance to boiling. Precipitation in large or very large flakes. Coagulates on boiling. Spontaneous coagulation after 3 to 4½ hours. Odor acid, but flavor still sweet</td></tr>
<tr><td>7° Yellow brown</td><td>14° S. = 31° D.</td></tr>
<tr><td colspan="2">The milk has passed the limit of resistance to boiling. Precipitates large flakes. Does not resist boiling. Spontaneous coagulation in 1½ to 3 hours. Odor and taste acid</td></tr>
<tr><td>8° Yellow</td><td>16° S. = 36° D.</td></tr>
<tr><td colspan="2">The milk has approached spontaneous coagulation. Coagulates in very large flakes. Coagulates on moderate heating. Coagulates spontaneously after 1½ hours as maximum. Odor and taste acid</td></tr>
<tr><td>9° Red (dark)</td><td>7° to 8° S. = 16° to 18° D.</td></tr>
<tr><td colspan="2">Coagulation: advanced cheesy. Coagulates in very large flakes. Coagulates at first heating and very quickly spontaneously. Taste—sweet. Merchandising value very doubtful</td></tr>
<tr><td>10° Violet</td><td>8° to 9° S. = 18° to 20° D.</td></tr>
<tr><td colspan="2">Milk called "alkaline." Rich in alkaline salts. Precipitates in very fine flakes. The milk has been neutralized (salted). The constitution is abnormal and improper for consumption as a beverage. Improper for cheese making</td></tr>
</table>

# DAIRY NOTES

## POISONOUS ACIDS IN CONDENSED MILK

The presence in condensed milk of organic and mineral acids, in excess of the amount which normal fresh milk contains, is conducive to the formation of lumps.

Excessive amounts of acid in condensed milk may be the result of fermentations but usually are due to lack of good sugar, or the use of acid flux in making or sealing of the tin cans. The presence of lumps in milk usually indicates that the milk is either acid or gaseous. The acid curdles the casein in the milk, which is detrimental to the market value of the milk.

The most common channel through which condensed milk becomes contaminated is through the use of zinc chloride as a flux in the making and sealing of the cans. The zinc chloride precedes the solder in the sealing process and some of it is sure to sweat through the seams into the interior of the can, chemically uniting with the casein forming caseinate of zinc, which is highly poisonous. The presence of zinc chloride is noted by a pink or brownish red color of the milk.

In order to avoid the appearance of lumps in condensed milk from this source, cans should be used that are sealed with the flux that is acid free. Dry powdered resin, or resin dissolved in alcohol is harmless in this respect, and is just as effective as the acid fluxes.

Orma N. Smith,
*Ames, Iowa.*

## REPORT ON TWO OLD SAMPLES OF BUTTER

Two samples of tinned butter, at least twenty years old, had been kept in the office of the Dairy Commissioner, Ottawa, exposed summer and winter to room temperatures.

Examination on September 16 showed that both tins were in good condition, hermetically sealed and tight. There was no evidence of "swells" or other damage.

*No. 1.* (*Laboratory No. 74479*). Labelled "Pierre de Bacourt, Dorchester, Que. Extra Finest Canadian Butter." Packed dry in round tin. On opening the tin the butter was found to be soft, with no texture. In appearance it was oleogenous and curdled. Color: light (pale) -

yellow: Odor: slightly rancid, but largely yeasty and not very unpleasant. Very disagreeable taste and quite non-edible.

*No. 2. (Laboratory No. 74480).* Labelled "Pure Canadian Butter." Canadian Government Dairy Station, Calgary, Alta. Packed in brine in rectangular tin, in two 1-pound prints, each wrapped in parchment paper. No liquid on opening tin. Very strong rancid odor, slightly aromatic. Very soft, with curdled appearance and no texture. Color: pale yellow; white in spots or patches. Absolutely non-edible.

*Analysis*

| | NUMBER 1 | NUMBER 2 |
|---|---|---|
| Water | 14.87 | 33.60* |
| Fat | 79.75 | 57.24 |
| Curd | 1.42 | 3.03 |
| Ash† | 3.98 | 6.11 |
| | 99.98 | 100.00 |
| | 3.80 | 5.94 |

* This includes water absorbed from brine in which the butter was packed.
† Including salt.

*Analysis of butter fats*

| | NUMBER 1 | NUMBER 2 |
|---|---|---|
| Butyro-refractometer at 25°C | 48.98 | 43.44 |
| Iodine No | 30.70 | 31.62 |
| Reickert Meisl No | 31.10 | 25.45 |
| Polenski No | 2.03 | 2.75 |
| Acid No | 45.33 | 121.3 |
| Percentage of free acid in oleic | 22.45 | 60.08 |
| Soluble fatty acids as butyric | 5.25 | 4.11 |
| Insoluble fatty acids | 84.93 | 86.92 |
| Saponification No | 227.6 | 225.8 |

The refractometer readings are in each case lower than would be expected with normal butters. The lowering of the refractometer indices is explained by the abnormal content of free fatty acids (Lewkowitsch, Vol. I, p. 338).

The iodine numbers are normal.

The lower Reichert Meisl No. and higher Polenski No. for fat No. 2 would seem to indicate conversion of soluble volatile acids into insoluble volatile acids in the presence of a large amount of water. This deduction is further supported by the figures obtained for soluble and insoluble fatty acids.

The acid numbers are very high especially in No. 2, and indicate the effect on hydrolysis of glycerides produced by the presence of a large amount of water.

The saponification values are normal.

For comparison the following limits for normal butter are given (Lewkowitsch, Vol. I, p. 338).

| | |
|---|---|
| Butyro-refractometer at 25°C | 49.5–54.0 |
| Iodine No | 26.0–35.0 |
| Reichert-Meisl No | 24.0–29.4 |
| Polenski No | 2.4 |
| Acid No | 0.5 |
| Soluble fatty acids as butyric | 5.0 |
| Insoluble fatty acids | 87.0 |
| Saponification No | 224.0 |

FRANK L. SHUTT,
*Dominion Chemist, Department of*
*Agriculture, Ottawa, Canada.*

*Dairy Bacteriology.* PROFESSOR ORLA-JENSEN. Published by J. & A. Churchill, London.

Dr. Orla-Jensen, Professor of Technical Biochemistry in the Polytechnic College, Copenhagen, Denmark, has recently brought out a new addition of his Dairy Bacteriology which has been translated into English. This work is a fine addition to our text-books on Dairy Bacteriology. It aims to make practical application of bacteriological principles to dairy work. It is the outgrowth of the author's twenty-five years experience in bacteriological research.

Among the subjects covered are: Micro-organisms and Fermentations, Bacteria, Yeasts and Moulds, Cleaning and the Procurement of Milk, Preservation of Milk, Application of Lactic Acid Fermentation in the Dairy Industry, Microflora of Butter, The Ripening Processes of the Different Cheeses, The Grading of Milk.

J. H. F.

# ENERGY REQUIREMENTS OF DAIRY COWS

## I. THE QUANTITIES OF NUTRITIVE ENERGY NECESSARY TO MAINTAIN DAIRY COWS IN NUTRITIVE EQUILIBRIUM*

EDWARD B. MEIGS AND H. T. CONVERSE

*Research Laboratories, Bureau of Dairying, United States Department of Agriculture*

### INTRODUCTION

For the past eight years, work on the nutriton of dairy cows has been in progress at the Beltsville (Md.) experiment farm of the United States Department of Agriculture. In the carrying out of this work it has been necessary, as it always is in scientific research, to center on certain aspects of the subject. But the effort has been made to keep, at the same time, more or less in touch with the whole field, in the hope that it would be possible to make some progress toward working out rations which would be permanently satisfactory for dairy cows—rations, that is, on which a liberal milk yield with satisfactory general health and reproductive capacity could be maintained in a herd of dairy cows through an unlimited number of generations.

The working out of such rations brings up quantitative as well as qualitative questions; and, if one surveys this field of nutrition as it stands at the present time, one finds that only two of the numerous dietary factors have so far been investigated from the quantitative standpoint, namely, protein and nutritive energy.[1]

Recent developments in physiological chemistry have shown that there is, strictly speaking, no such thing as a quantitative requirement of protein for dairy cows. Different proteins differ widely in their chemical constitution, and the dairy cow requires much larger quantities of some of them for the manufacture of a given quantity of milk protein than of others.

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*Received for publication January 15, 1925.

[1] There is a tacit disagreement in the literature as to how the energy content of foods should be measured; some investigators give their feeding standards in terms of "total digestible nutrients"; others, in terms of "net energy." Throughout this article the phrase "nutritive energy" will be used to cover either of these conceptions.

With regard to nutritive energy, on the other hand, there is reason to believe that the quantity required by dairy cows under different conditions of milk and fat yield and of body weight can be at least approximately determined; and, as this question stands at the very center of any general plan for rationing these animals, records have been kept at Beltsville of the food eaten, of the milk and fat yield, and of the changes in body weight of the members of the herd, which, it is believed, will throw light on it. The results are to be regarded as supplementing those which have already been obtained by other investigators in the same field, and must be considered and discussed in close connection with them.

## EXISTING FEEDING STANDARDS

The experimental work on which the existing feeding standards are based is of three types. Haecker (3, 4, 5) and Eckles (2) determined the quantities of food necessary to keep milking cows at uniform body weight. Armsby bases his standard on an experiment of Kellner in which the complete energy balance was determined for a milking cow (1, p. 493–500, 511–516).[2] Savage (7) raises the question: What will happen if cows are fed more than is necessary, according to Haecker's results, to keep them at uniform body weight. For the present, the question raised by Savage will be left aside, and the discussion will be confined to the question how much food is necessary to keep milking cows in nutritive equilibrium or at uniform body weight.

Even at this early stage of the subject one encounters more than one tacit difference of opinion among the different investigators. Eckles used Armsby's tables to obtain the net energy content of the feeds required by his cows for the maintenance of uniform body weight and gave his standard for the energy requirements of dairy cows in terms of net energy. Some years later, Armsby published a feeding standard for dairy cows. The energy requirements in Armsby's standard are given in terms of net energy, and are based, as was stated above, on one of Kellner's experiments. If one compares Armsby's standard with Eckles's, one finds that the former requires from 11 to 23 per cent less net energy than the latter for a given amount of milk.

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[2] Although Armsby considers the results obtained by Kellner with three cows, he bases his standard on the performance of the most economical of the three, rather than on the average.

In Eckles's experiments the food intake, milk and fat yield, and body weight were followed in eight milking cows for a year each, and in two others for 110 and 120 days respectively; in the experiment of Kellner, on which Armsby's standard is based, the complete energy balance was determined in the case of a single milking cow for a period of two weeks. It may be said, on the one hand, in explanation of the disagreement between the two standards, that the maintenance of uniform body weight is not always a very sure indication that an animal is in nutritive equilibrium; on the other hand, it may be said that it is a little unsafe to base a feeding standard which is to be used in practice on the results of a single physiological experiment.

Changes in body weight in mammals are caused chiefly by changes in the quantity of muscle and fat contained in their bodies. Changes in other tissues are likely to be so small as to be masked by changes occurring in the two above mentioned. Muscle is a tissue which contains from seventy to eighty per cent of water; its dry matter is chiefly protein. Fat, on the other hand, contains very little water, and its dry matter has about 2¼ times as much nutritive energy per gram as protein. It may be considered, therefore, that a gram of fat tissue has from eight to ten times as much nutritive energy as a gram of muscle tissue. If an animal is fed a ration which contains a surplus of protein (as in Eckles's experiments), though a slight deficit in nutritive energy, it is quite conceivable that it might build muscle tissue from the protein surplus and supply the deficit in nutritive energy by using a small quantity of its own body fat. Under these circumstances, the animal might gain weight though being in negative energy balance. It has been shown experimentally that this process actually does occur in young animals when they are fed rations deficient in nutritive energy (1, p. 280).

The fact that an animal is gaining weight does not, therefore, necessarily show that it is receiving sufficient nutritive energy in its rations. But if a healthy animal, which is getting a surplus of protein and of the other chemical compounds necessary for complete nutrition, is losing weight, this does show that it is not receiving sufficient nutritive energy in its rations. For the surplus of protein and other nutritive compounds will prevent it from losing muscle tissue, except so far as such loss may be necessary to supply the energy deficiency; and, if this deficiency does not exist, neither muscle nor fat tissue will be lost.

The case may be summed up by saying that if a healthy animal which is receiving a well-balanced ration with a liberal protein content loses weight, the fact constitutes strong evidence for believing that the ration used is deficient in nutritive energy. Conversely, a well-balanced ration high in protein which is just sufficient to maintain a healthy animal at uniform body weight certainly does not contain a surplus of nutritive energy. In the case of a short-time experiment with a young animal, such a ration might be somewhat deficient in nutritive energy for the reasons above mentioned, but the process of building muscle tissue at the expense of body fat could not go on very long. There is every reason to believe, therefore, that uniform body weight maintained for a year or more is a very sure indication that the animal under consideration remained nearly in nutritive equilibrium throughout the period of the experiment. If the animal under consideration was mature and farrow, and had, therefore, no tendency to build watery tissues at the expense of body fat, the reasons for thinking that it was in nutritive equilibrium are still stronger. It seems to us, therefore, that such experiments as those of Eckles[3] furnish a very good basis for judging the average quantities of food necessary to keep milking cows in nutritive equilibrium under practical conditions.

## RESULTS OBTAINED AT THE BELTSVILLE FARM ON THE QUANTITY OF NUTRITIVE ENERGY NECESSARY TO MAINTAIN UNIFORM BODY WEIGHT

The records which have been kept at the Beltsville farm for the last four years make it possible to throw still more light on the relation between food intake and change in body weight in milking cows. These records consist of figures for the monthly milk and fat yield, food consumption, and change in body weight for every member of the herd. It is possible from these records to calculate the monthly intake of either net energy or total nutrients for any cow and to compare it with her requirements according to any of the feeding standards.[4] It is easy then to compare the surplus or deficit in nutritive energy consumed with the monthly change in body weight, and, in this way, to determine which of the standards comes nearest to indicating the quantity of nutritive energy required to maintain uniform body weight.

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[3] The cows used by Eckles were mature and farrow.

The body weight of the cows at Beltsville has been determined by weighing them on three successive days at the same time each day. The average of these three weights is taken as the body weight of the cow at the period of the weighings. Body weights were usually taken at the beginning of each calendar month; in calculating the maintenance requirements for any given month, the average between the figure obtained at the beginning of the month and that obtained at the beginning of the next succeeding month was taken.

In the Armsby, Eckles, and Savage feeding standards, the maintenance requirements are given for 1000 pound cows. In calculating the requirements for cows that weighed more or less than a thousand pounds we used corrections based on the assumption that maintenance requirements are proportional to body surface, our requirements being calculated from the formula

$$X = M\left(\frac{W}{1000}\right)^{\frac{2}{3}}$$

where $X$ is the maintenance requirement to be determined; $M$, the maintenance requirement of a 1000-pound cow; and $W$, the body weight of the cow whose requirement is to be determined.

For the purposes of this study the cows at the Station may be divided into two or three different classes. Each year a number of them are run on yearly official test. They are kept in box stalls, milked three times a day, and fed very liberally on a ration with a rather narrow nutritive ratio, not only while they are milking, but also before they calve. Another set of cows has been used in experiments which required that they should be fed as much as they would eat while milking. They also received a ration with a rather narrow nutritive ratio. They were fed liberally before they calved, though not so high as the test cows; and they were kept in stanchions and milked only twice a day. Still a third set of cows has been kept under the ordinary herd routine. They have been kept in stanchions and milked only twice a day; and, while milking, were fed decidedly less liberally than either of the other sets. Before calving, they were fed about as liberally as the second-mentioned group. Their ration had a somewhat wider nutritive ratio than that of the other two sets. In making the study at present under discussion, the herd cows were put in one group, and all the others in a second group. As young cows and pregnant cows are likely to be building watery tissues at the expense of body fat, only those animals have been used which were over five years old and farrow.

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[4] Of course none of the cows used in these studies had any pasture.

It has been thought worth while to compare consumption with requirement and change in body weight according to the Armsby, Eckles, and Savage feeding standards;[5] and the data contained in the records have been handled in the following manner. The quantity of net energy or of total digestible nutrients required by each cow in each calendar month for her maintenance and milk and fat yield, according to each of the above-mentioned standards, has been calculated and subtracted from her consumption of either net energy or total digestible nutrients. The percentage consumed above or below the requirement was then calculated. For each of the standards, then, each group of cows, as above described, was divided into two subgroups, namely, those that ate less than they required, and those that ate more. The average percentage consumed above or below requirement, for each of these groups, was then calculated and compared with the average corresponding monthly change in body weight.

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[5] In calculating the net energy content of dairy feeds, we have throughout used the figures given in Table VII in Armsby's "Nutrition of Farm Animals" (1, p. 715–721). In calculating the total digestible nutrients, we have used the figures from Table III of Henry and Morrison's "Feeds and Feeding" (6, p. 653–666). In calculating requirements for maintenance and milk yield we have used Table I, p. 711, and Table V, p. 714, of Armsby's book (1), and the tables given on p. 133 of "Feeds and Feeding" (6).

The results of these calculations are given in tables 1 and 2. It will be seen from the figures given in the tables that there was a marked general tendency for the cows to lose weight when they were eating less than the requirements of either the Eckles or the Savage standard, and a very marked tendency for them to lose weight when they were consuming less than the Armsby standard required. But a study of the figures for the individual cow-months shows that these are rather irregular.

It was hoped that our procedure for determining body weight described above would give a fairly good idea of any change that might take place in the weight of the body tissues. The sources of error in this kind of study are changes in the contents of the intestinal tract and the bladder, and changes in the water content of the body tissues. It would seem not unlikely, a priori, that by the procedure above outlined one would get figures which, when corrected by subtracting a certain constant to represent the contents of the intestine and of the bladder, would represent the true weight of the body tissues with their average water content.

A study of the manner in which the body weight of the individual cows varies from month to month shows, however, that this is not always the case. It is not uncommon to find that a cow kept for two or three months on a deficient ration will lose perhaps a hundred pounds in the first part of the period, according to our method of determining the body weight, and then regain half of the previous loss, though still on the same ration. It would seem, therefore, that an abnormally high or low intestinal content or water content of the body tissues may persist for several days, and even our three-day average weights can not be taken as a very sure indication of the weight of the body tissues plus a constant.

These irregularities are readily smoothed out, however, if

## TABLE 1

*Relation between change in body weight and percentage of net energy or total digestible nutrients consumed above or below the requirements of Armsby, Eckles, and Savage feeding standards*

Most of the cows used were under test conditions; all of them were fed very liberally while milking.

### ARMSBY STANDARD

| | Percentage net energy consumed below requirement | Pounds body weight gained or lost in month |
|---|---|---|
| Cows eating less than Armsby requirement: individual cases | 15.5 | −13 |
| | 3.8 | −30 |
| Ave. | 9.6 | −21.5 |

| | Percentage net energy consumed above requirement | Pounds body weight gained or lost in month |
|---|---|---|
| Cows eating more than Armsby requirement: individual cases (continued) | 19.3 | +13 |
| | 19.4 | −5 |
| | 20.8 | +27 |
| | 22.1 | −14 |
| | 22.3 | +32 |
| | 23.0 | −1 |
| | 23.1 | +19 |
| | 23.2 | +11 |
| | 23.2 | +22 |
| | 23.5 | +41 |
| | 23.7 | −21 |
| | 24.1 | +1 |
| | 24.8 | +4 |
| | 25.4 | −31 |
| | 25.6 | 0 |
| | 25.6 | +75 |

### ECKLES STANDARD

| | Percentage net energy consumed below requirement | Pounds body weight gained or lost in month |
|---|---|---|
| Cows eating less than Eckles requirement: individual cases | 22.1 | −13 |
| | 11.2 | −30 |
| | 8.7 | −118 |
| | 8.1 | −8 |
| | 5.6 | −39 |
| | 4.8 | +23 |
| | 2.8 | 0 |
| Ave. | 9.0 | −26.4 |

| | Percentage net energy consumed above requirement | Pounds body weight gained or lost in month |
|---|---|---|
| Cows eating more than Eckles requirement: individual cases (continued) | 9.1 | +4 |
| | 9.5 | +2 |
| | 9.8 | +13 |
| | 9.9 | +11 |
| | 10.3 | +22 |
| | 11.0 | +25 |
| | 11.8 | +75 |
| | 11.9 | +1 |
| | 12.6 | +32 |
| | 13.0 | +41 |
| | 13.6 | 0 |
| | 14.5 | +19 |
| | 14.7 | +14 |
| | 15.2 | −21 |
| | 15.5 | −1 |
| | 16.2 | −31 |

### SAVAGE STANDARD

| | Percentage total digestible nutrients consumed below requirement | Pounds body weight gained or lost in month |
|---|---|---|
| Cows eating less than Savage requirement: individual cases | 27.6 | −13 |
| | 17.9 | −30 |
| | 15.3 | −8 |
| | 13.1 | −118 |
| | 9.5 | +35 |
| | 8.6 | +23 |
| | 7.7 | −39 |
| | 7.5 | −23 |
| | 7.3 | 0 |
| | 4.3 | +51 |
| | 1.3 | −32 |
| | 0.6 | +4 |
| | 0.3 | −1 |
| | 0.2 | −6 |
| Ave. | 8.7 | −11.2 |

| | Percentage total digestible nutrients consumed above requirement | Pounds body weight gained or lost in month |
|---|---|---|
| Cows eating more than Savage requirement: individual cases | 0.8 | +11 |
| | 0.8 | +22 |
| | 0.9 | +14 |
| | 1.2 | +75 |
| | 1.5 | +2 |
| | 1.7 | −1 |
| | 2.6 | +1 |
| | 3.0 | +25 |
| | 3.8 | +13 |
| | 4.3 | +27 |
| | 4.5 | +32 |
| | 5.0 | −14 |
| | 5.0 | −5 |
| | 5.8 | 0 |
| | 6.0 | −21 |
| | 6.2 | +14 |

| | Percentage net energy consumed above requirement | Pounds body weight gained or lost in month |
|---|---|---|
| Cows eating more than Armsby requirement: individual cases | 1.7 | −8 |
| | 1.8 | −118 |
| | 5.0 | +23 |
| | 5.8 | 0 |
| | 8.7 | −39 |
| | 11.6 | +35 |
| | 13.3 | +51 |
| | 14.3 | −23 |
| | 15.8 | −6 |
| | 15.9 | −1 |
| | 17.4 | −32 |
| | 17.8 | +14 |
| | 18.3 | +2 |
| Cows eating more than Armsby requirement: individual cases (continued) | 26.2 | +9 |
| | 26.2 | +25 |
| | 27.0 | +9 |
| | 27.3 | +14 |
| | 27.4 | −5 |
| | 28.8 | +26 |
| | 29.0 | +16 |
| | 29.1 | −1 |
| | 30.5 | +23 |
| | 30.7 | −5 |
| | 31.4 | −3 |
| | 31.6 | −6 |
| | 31.8 | +25 |
| | 32.1 | +10 |
| | 32.2 | −52 |
| | 32.5 | −32 |
| | 32.8 | +21 |
| | 32.9 | +21 |
| | 34.1 | +36 |
| | 41.0 | +21 |
| | 41.5 | +33 |
| Ave. | 23.5 | +5.1 |
| Cows eating more than Eckles requirement: individual cases | 1.2 | +35 |
| | 1.3 | −6 |
| | 2.0 | −23 |
| | 2.0 | +51 |
| | 2.1 | −5 |
| | 3.3 | −32 |
| | 4.2 | +9 |
| | 5.0 | +27 |
| | 6.1 | −14 |
| | 6.7 | −1 |
| | 7.3 | +9 |
| | 7.6 | +14 |
| | 8.3 | −1 |
| Cows eating more than Eckles requirement: individual cases (continued) | 16.6 | +16 |
| | 16.9 | −5 |
| | 17.8 | +10 |
| | 18.7 | +26 |
| | 19.2 | −6 |
| | 19.6 | −32 |
| | 19.9 | +36 |
| | 20.0 | −5 |
| | 20.5 | +25 |
| | 21.1 | −52 |
| | 21.8 | +23 |
| | 22.4 | −3 |
| | 22.7 | +21 |
| | 22.9 | +21 |
| | 28.8 | +33 |
| | 29.8 | +21 |
| Ave. | 13.6 | +8.8 |
| Cows eating more than Savage requirement: individual cases (continued) | 6.5 | +10 |
| | 6.5 | +19 |
| | 7.1 | +41 |
| | 8.1 | +16 |
| | 8.2 | −32 |
| | 8.5 | −3 |
| | 8.6 | +9 |
| | 8.8 | +36 |
| | 9.0 | −31 |
| | 9.7 | −5 |
| | 10.9 | +25 |
| | 11.3 | +23 |
| | 11.6 | +26 |
| | 11.7 | +21 |
| | 12.0 | +9 |
| | 12.2 | −52 |
| | 12.7 | −1 |
| | 13.0 | −6 |
| | 14.4 | −5 |
| | 14.4 | +21 |
| | 17.8 | +33 |
| | 18.8 | +21 |
| Ave. | 7.8 | +9.7 |

## TABLE 2

*Relation between change in body weight and percentage net energy or total digestible nutrients consumed above or below the requirements of the Armsby, Eckles, and Savage feeding standards*

The cows used were under herd conditions and not very liberally fed while milking.

| | ARMSBY STANDARD | | | | |
|---|---|---|---|---|---|
| | Percentage net energy consumed below requirement | Pounds body weight gained or lost in month | | Percentage net energy consumed above requirement | Pounds body weight gained or lost in month |
| Cows eating less than Armsby requirement: individual cases | 29.1 | −118 | Cows eating more than Armsby requirement: individual cases | 0.2 | −37 |
| | 28.3 | −71 | | 5.6 | −30 |
| | 18.2 | −56 | | 9.0 | +5 |
| | 17.5 | −44 | | 9.3 | −37 |
| | 8.8 | −74 | | 9.8 | +74 |
| | 8.1 | −52 | | 12.5 | +18 |
| | 5.3 | +19 | | 14.3 | +15 |
| | 2.0 | +48 | | 17.6 | −29 |
| Ave. | 14.7 | −43.5 | | 17.8 | +32 |
| | | | | 19.9 | −17 |
| | | | | 25.0 | +29 |
| | | | | 25.1 | +11 |
| | | | | 26.0 | +5 |
| | | | | 29.2 | +22 |
| | | | | 32.6 | +9 |
| | | | | 39.7 | +7 |
| | | | Ave. | 18.3 | +4.8 |

| | ECKLES STANDARD | | | | |
|---|---|---|---|---|---|
| | Percentage net energy consumed below requirement | Pounds body weight gained or lost in month | | Percentage net energy consumed above requirement | Pounds body weight gained or lost in month |
| Cows eating less than Eckles requirement: individual cases | 36.2 | −71 | Cows eating more than Eckles requirement: individual cases | 3.7 | +18 |
| | 33.7 | −118 | | 4.2 | −29 |
| | 25.1 | −44 | | 5.7 | −17 |
| | 24.1 | −56 | | 6.0 | +15 |
| | 23.4 | −74 | | 7.2 | +32 |
| | 19.5 | −52 | | 8.4 | +5 |
| | 15.0 | +48 | | 10.7 | +29 |
| | 12.2 | +19 | | 11.0 | +11 |
| | 8.4 | −37 | | 19.6 | +22 |
| | 3.3 | −30 | | 20.2 | +9 |
| | 2.9 | −37 | | 21.7 | +7 |
| | 1.0 | +5 | Ave. | 10.8 | +9.3 |
| | 0.7 | +74 | | | |
| Ave. | 15.8 | −28.7 | | | |

| | SAVAGE STANDARD | | | | |
|---|---|---|---|---|---|
| | Percentage total digestible nutrients consumed below requirements | Pounds body weight gained or lost in month | | Percentage total digestible nutrients consumed above requirement | Pounds body weight gained or lost in month |
| Cows eating less than Savage requirement: individual cases | 38.1 | −118 | Cows eating more than Savage requirement: individual cases | 0.6 | −29 |
| | 37.8 | −71 | | 1.6 | −17 |
| | 30.7 | −56 | | 3.9 | +11 |
| | 28.3 | −44 | | 6.2 | +29 |
| | 25.1 | −74 | | 7.3 | +5 |
| | 18.1 | −52 | | 7.8 | +22 |
| | 18.1 | +19 | | 12.1 | +7 |
| | 14.9 | +48 | | 12.4 | +9 |
| | 13.5 | −37 | Ave. | 6.5 | +4.6 |
| | 12.1 | −30 | | | |
| | 7.5 | −37 | | | |
| | 6.0 | +5 | | | |
| | 5.5 | +74 | | | |
| | 4.4 | +18 | | | |
| | 3.0 | +15 | | | |
| | 2.0 | +32 | | | |
| Ave. | 16.6 | −19.2 | | | |

enough figures are taken and averaged. For the present, the question of how many figures are necessary to secure a satisfactory average will be left aside, and the results as they have been obtained will be given and discussed.

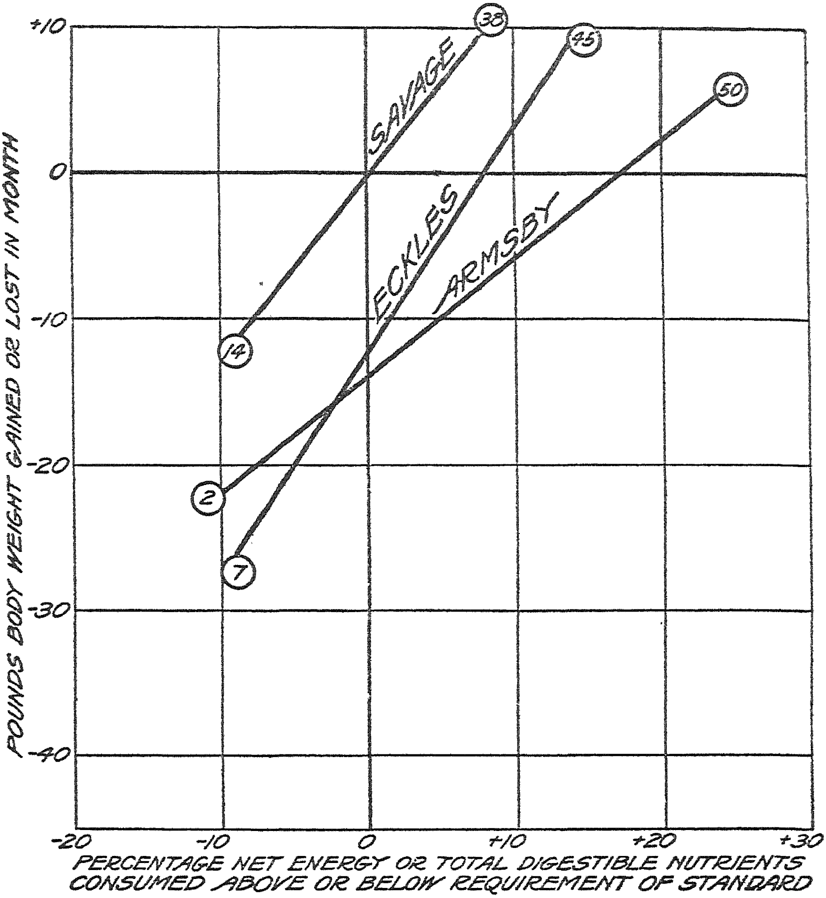

FIG. 1. RELATIONS BETWEEN CHANGE IN BODY WEIGHT AND PERCENTAGE OF NET ENERGY OR TOTAL DIGESTIBLE NUTRIENTS CONSUMED ABOVE OR BELOW REQUIREMENTS OF ARMSBY, ECKLES AND SAVAGE STANDARDS
Graphs plotted from data in table 1

The results given in tables 1 and 2 may be plotted as in figures

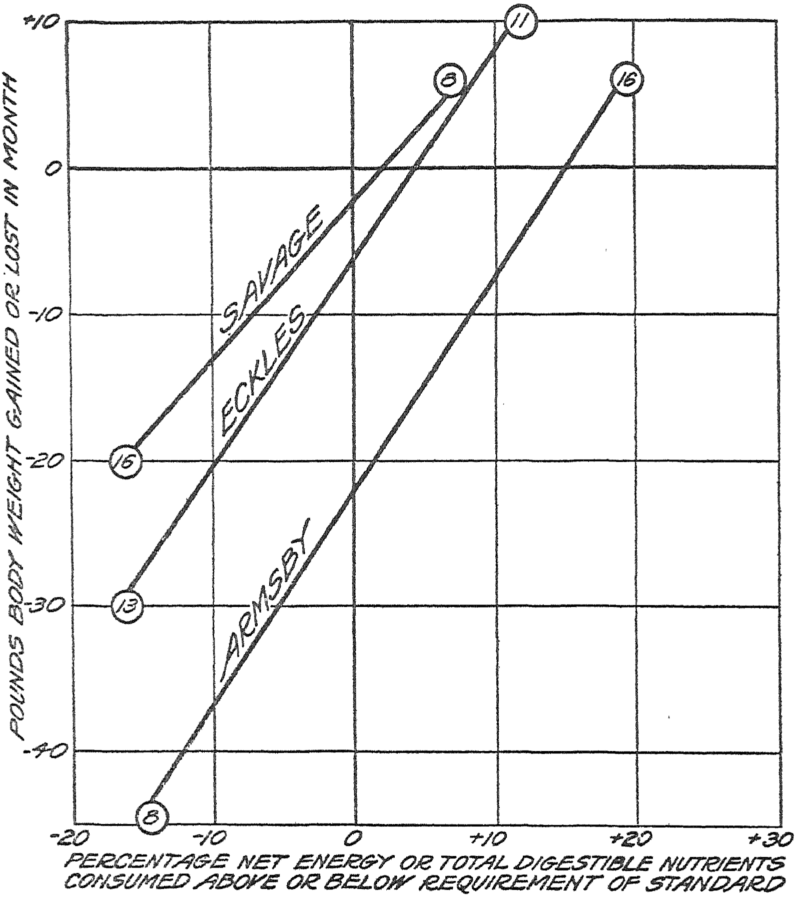

FIG. 2. RELATIONS BETWEEN CHANGE IN BODY WEIGHT AND PER CENT OF NET ENERGY OR DIGESTIBLE NUTRIENTS CONSUMED ABOVE OR BELOW REQUIREMENTS OF ARMSBY, ECKLES AND SAVAGE STANDARDS

Graphs plotted from data in table 2

1 and 2, the percentage of nutritive energy consumed above or below the requirements being used as abscissa, and the number of pounds gained or lost in the month, as ordinate.[6] In the figures, the points representing the change in body weight for each of the given percentages of nutritive energy consumed above or below requirement have been joined by straight lines. The figures inclosed by small circles give the number of cases on which each point in the chart is based. The point where the straight line for each standard crosses the line of zero change in weight may be taken as approximately indicating the percentage of nutritive energy above or below the requirement of each standard which the cows used in these experiments needed to maintain them at uniform body weight. The high-fed cows, according to these results, needed just the quantity of total digestible nutrients which would be allowed by the Savage standard, 8 per cent more net energy than would be allowed by the Eckles standard, and 17 per cent more net energy than would be allowed by the Armsby standard. The less highly fed cows required 2 per cent more than would be allowed by the Savage standard, 4.5 per cent more than would be allowed by the Eckles standard, and 15 per cent more than would be allowed by the Armsby standard. The agreement between the two sets of results seems to us remarkably close, in view of the small number of cases on which some of the points in the charts are based. Several circumstances indicate, however, that the number of cases on which these points are based is really sometimes too small to give entirely satisfactory results. Among these, the widely different slants for the lines for the various standards are particularly noticeable. In figure 3, the two sets of results have been combined, and present a

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[6] The data given in tables 1 and 2 may, of course, be handled in several other ways. For instance, the 15 or 20 cows which, on the average, maintained uniform body weight on rations near the requirement of each of the standards may be selected, and their average percentage consumption above or below the requirement of each standard may be calculated. The writer has tried several different ways of handling the data and found that they all lead to the conclusion that cows maintain uniform body weight when fed approximately according to the Eckles or Savage standard, and lose weight fairly rapidly, when fed according to the Armsby standard.

more convincing appearance. According to the results presented in this figure, the cows from which these records were obtained would have been kept at uniform body weight by a ration just

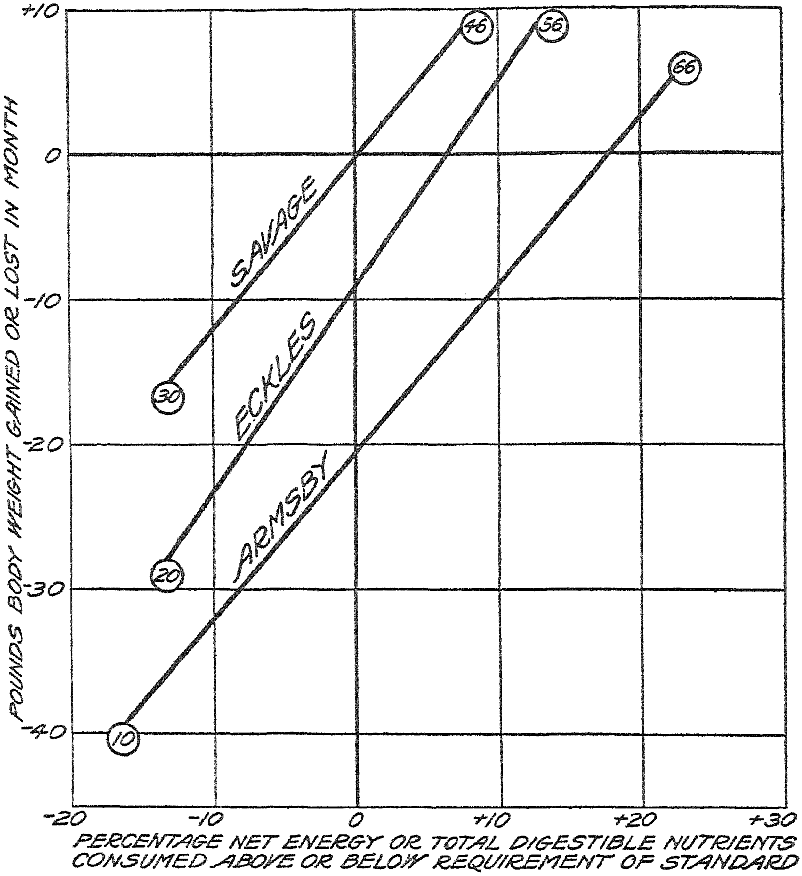

Fig. 3. Relations Between Change in Body Weight and Percentage of Net Energy or Total Digestible Nutrients Consumed above or Below Requirements of Armsby, Eckles and Savage Standards

Graphs plotted from combined data of tables 1 and 2

equal to that allowed by the Savage standard, 6.5 per cent more than that allowed by the Eckles standard, and 18 per cent more than that allowed by the Armsby standard.

Eckles' results and ours taken together point strongly to the conclusion that milking cows fed according to the Armsby standard would receive considerably less nutritive energy than was necessary to keep them at uniform body weight; for reasons which have already been given, the results point also to the conclusion that they would not receive enough to keep them in energy equilibrium. Just why the results obtained under practical conditions should disagree with the experiment of Kellner on which Armsby's standard is based can not be fully decided from the evidence at present in existence. It seems to us not unlikely, however, either that the cow used by Kellner may have secreted milk with more than average economy, or that there may have been some error in interpreting the results. In any case, it is clear that the extensive results obtained under practical conditions are the safer guide for the feeding of cows in practice.

## INDICATIONS REGARDING THE RELATIVE PRACTICAL USEFULNESS OF NET ENERGY VALUES AND TOTAL DIGESTIBLE NUTRIENTS TO BE GAINED FROM THE RESULTS SO FAR REPORTED

The quantities of net energy and of total digestible nutrients consumed by the cows used in Eckles's experiments have been calculated and compared with their requirements according to the Eckles and Savage standards respectively.[7] These cows consumed, on the average, 1.7 per cent more net energy than they required according to the Eckles standard, and 2.1 per cent less total digestible nutrients than they required according to the Savage standard.

Eckles's results and ours agree more closely, therefore, in regard to the total digestible nutrients necessary to maintain uniform body weight than in regard to the net energy. Whereas his cows required 2.1 per cent, less total digestible nutrients than ours to maintain uniform body weight, they required 6.5 minus 1.7, or 4.8 per cent less net energy. As the quantities of total digestible nutrients in different feeding stuffs do not run parallel to their net energy values, it becomes of interest to inquire into the respective proportions of total digestible nutrients and of net energy in Eckles's feeds and in ours. The results furnish a preliminary test as to whether total digestible nutrients or net energy values are a better measure of the relative values of different feeds for maintaining uniform body weight.

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[7] In calculating the total digestible nutrients and the net energy contained in the foods consumed by Eckles's cows, we have used the tables of Henry and Morrison (6) and of Armsby (1), respectively. See above.

The total feed consumed by Eckles's cows contained 42,044 therms net energy and 48,822 pounds total digestible nutrients; the ratio between therms net energy and pounds total digestible nutrients in his feeds was therefore $\frac{42,044}{48,882}$ or 0.861. Similarly, our cows consumed 48,140 therms net energy and 53,227 pounds total digestible nutrients, and the corresponding ratio in the case of our cows was 0.904. For every hundred pounds of total digestible nutrients, therefore, Eckles's cows consumed 86.1 therms net energy, while our cows consumed 90.4 therms net energy, or about 5 per cent more than his. It will be recollected that our cows had to consume only 2.1 per cent more total digestible nutrients to keep them at uniform body weight than Eckles's cows and 4.8 per cent more net energy. The results, therefore, point to the conclusion that total digestible nutrients are a better index of the relative values of feeds for maintaining uniform body weight in dairy cows than are net energy values. While the two sets of results are only 2.1 per cent apart when the total digestible nutrient content of the two sets of feeds are considered, they are 4.8 per cent apart when the net energy values are considered; and this latter discrepancy is fairly satisfactorily explained from a consideration of the respective quantities of total digestible nutrients and of net energy contained in the two sets of rations.

There is further reason to think that the quantity of total digestible nutrients necessary to keep dairy cows at uniform body weight is surprisingly constant when it is determined, as in Eckles's experiments and ours, for a number of cows over considerable periods and calculated according to the requirements of the Savage standard.

Haecker, like Eckles, carried out experiments in which the quantities of feed necessary to keep milking cows at uniform body weight were determined. Haecker's experiments differed from Eckles's, however, in that his cows were not kept farrow, and that some of them were not mature. As the cows used by Haecker were not kept on the experiments for as long as 200 days consecutively, it is probable that some of them would have shown a tendency to build muscle tissue at the expense of body fat, and might, therefore, have gained weight to some extent on a barely sufficient ration, or maintained uniform weight on a somewhat deficient ration.

In spite of these possibilities, we have thought it worth while to compare the total digestible nutrients[8] consumed by Haecker's cows with their requirements for maintenance and milk yield according to the Savage standard. The results show that these cows had to consume, on the average, 5 per cent less total digestible nutrients than the Savage standard calls for in order to maintain uniform body weight.

Haecker's cows required somewhat less digestible nutrients to keep them at uniform body weight than did either Eckles's or ours, as was to have been expected from the fact that they were not always either mature or farrow. It will be remembered that Eckles's cows required 2.1 per cent less than the Savage standard; and ours, just the requirement of the Savage standard. The greatest difference, therefore, lies between our results and Haecker's, and amounts to 5 per cent.

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[8] In estimating the total digestible nutrients consumed by Haecker's cows, we have used the figures which he obtained from his own analyses of the feeds used by him. As far as it was possible, however, from the data given by Haecker, we have calculated the quantities of total digestible nutrients in the food consumed by his cows according to the figures given by Henry and Morrison in the 1921 edition of "Feeds and Feeding" (6). In most cases there is a very close agreement between these results and those obtained by Haecker from his own analyses; where a difference exists, it has been of such a nature as to indicate that the agreement between Haecker's results, on the one hand, and those of Eckles and ourselves, on the other, in regard to the quantity of total digestible nutrients necessary to maintain uniform body weight in milking cows would be closer if the total digestible nutrients in Haecker's feeds were calculated according to the figures of Henry and Morrison than it is when they are calculated from the results of his own analyses. See (5).

This remarkable agreement between three independent sets of results carried out under different climatic conditions, with different kinds of cows, and with different kinds of feed, shows that the figures given by Henry and Morrison for total digestible nutrients furnish a system for estimating the relative usefulness of feeds as sources of nutritive energy which is, to say the least, very fairly accurate. And, as the disagreement between our results and Eckles's in terms of net energy value is larger than in terms of total digestible nutrients it is proper to inquire whether the latter system is not just as good or better for practical purposes than the former. It is, of course, fully realized that the considerations which have just been given are not sufficient to answer finally so important a question as this. Experiments to throw further light on it are now being planned at Beltsville, and the results will be published as soon as possible after they have been completed. In the meantime, however, it is proposed to inquire critically into the physiological considerations underlying the use of the two sets of units, and into the experimental basis for the figures which exist at present in the literature. This will be done in the next article of this series.

## SUMMARY

Recent results obtained at the Beltsville experiment farm are in close agreement with the extensive investigations of Haecker in regard to the quantities of total digestible nutrients required to keep milking cows at uniform body weight. When the results obtained by Eckles in the same field are calculated in terms of total digestible nutrients, they also are found to be in close agreement with the two sets of results above mentioned.

The results obtained by Eckles do not agree with Armsby's feeding standard in regard to the net energy requirements of milking cows. Our own results are decidedly more nearly in agreement with Eckles on this question than with Armsby. A study of the Armsby standard shows that it is not based on any experiments carried out under conditions approaching those which obtain in practice, but on a train of physiological reasoning which rests on an experiment of Kellner in which the complete

energy balance was obtained in one milking cow for a period of two weeks. Although two other cows were considered, both were somewhat less economical, and were not used in the calculations.

The agreement between Eckles's results and ours is closer in terms of total digestible nutrients than in terms of net energy. It is proper, therefore, to make a critical examination into the physiological considerations underlying the determination of net energy values and into the experimental basis for the net energy figures which exist at present in the literature.

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# A COMPARATIVE STUDY OF METHODS FOR DETERMINING THE PER CENT OF BUTTERFAT IN DAIRY PRODUCTS*

## II. ICE CREAM

RICHARD C. FISHER AND CHARLES C. WALTS

*From the Department of Dairy Husbandry, Storrs Agricultural Experiment Station, Storrs, Connecticut*

The first paper (1) on this subject deals with a comparison of the Mojonnier, the Modified Babcock and the Troy-Fucoma method for determining the per cent of butterfat in ice cream. Observation of the Troy-Fucoma method in these first trials frequently showed a hazy film in the fat column. This film usually disappeared when samples were centrifuged in a heated tester and run for a few minutes longer than the prescribed four minutes. It was also observed that results obtained tended to be higher when the centrifuging period was extended beyond four minutes.

Our next efforts therefore have been confined to a study as to the influence of the following factors:

1. Influence of the length of centrifuging on the per cent of fat and the appearance of the fat column.
2. Influence of temperature of tester and fat column on appearance of the fat column and the final per cent of fat.
3. Accuracy of the Torsion Balance for weighing the charge.

### INFLUENCE OF LENGTH OF CENTRIFUGING AND HEATED TESTER

The percentage of fat as obtained by duplicate determinations on the Mojonnier tester were used as a standard of comparison in each instance. Several duplicate samples of ice cream were prepared according to Troy-Fucoma directions, weighing being done with a Torsion cream balance into previously calibrated

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* Received for publication March 1, 1925.

test bottles.[1] These samples were then centrifuged for different periods of time. The per cent of fat and appearance of the fat column was noted in each case and especially the presence or absence of the characteristic hazy film at the bottom of the fat column. Results are reported in table 1. A study of the data in table 1 seems to indicate that centrifuging for four minutes or 2 times two minutes is not a sufficient length of time to remove the hazy film or to bring all of the fat up into the fat column. Thus after four minutes centrifuging, there was a pronounced film and the results in the majority of the instances were lower than the Mojonnier.

TABLE 1

*Influence of length of time of centrifuging on the per cent of fat*

| MOJONNIER TEST | PER CENT OF FAT OBTAINED WHEN CENTRIFUGED FOR | | | | | | INCREASE IN PER CENT OF FAT OVER FIRST FUCOMA READING | |
|---|---|---|---|---|---|---|---|---|
| | Four minutes* | | 2 × two minutes† | | 3 × two minutes‡ | | 2 × two minutes | 3 × two minutes |
| C. A. C. ice cream mix, 10/4/24<br>13.64 to 13.71, average 13.675 | 13.6 | Film prominent | 13.7 | Film prominent | 13.9 | Film inconspicuous | 0.1 | 0.3 |
| | 13.8 | | 13.8 | | 14.0 | | 0.0 | 0.2 |
| | 13.4 | | 13.7 | | 13.9 | | 0.3 | 0.5 |
| | 13.8 | | 13.8 | | 14.0 | | 0.2 | 0.2 |
| | 13.0 | | 13.4 | | 13.8 | | 0.4 | 0.8 |
| | 13.9 | | 13.9 | | 14.2 | | 0.0 | 0.3 |
| | 13.6 | | 13.7 | | 13.9 | | 0.1 | 0.7 |
| | 13.2 | | 13.6 | | 13.9 | | 0.4 | 0.7 |
| | 13.0 | | 13.4 | | 13.8 | | 0.4 | 0.8 |
| | 13.0 | | 13.2 | | 13.6 | | 0.2 | 0.6 |
| | 13.2 | | 13.4 | | 13.7 | | 0.2 | 0.5 |
| | VARIATION FROM AVERAGE MOJONNIER TEST | | | | | | VARIATIONS FROM FIRST FUCOMA READINGS | |
| | −0.166% | | −0.015% | | +0.206% | | 0.2% | 0.5% |

* Four minutes = bringing tester up to speed and allowing to run at full speed for four minutes.

† Two × two minutes = bringing tester up to speed, running for two minutes with steam off, then bringing up to speed again and running for two minutes with steam off.

‡ Three × two minutes = bringing tester up to speed three times and allowing it to run for two minutes each time with steam off.

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[1] Calibration of Fucoma test bottles was made possible through the courtesy of Dr. E. M. Bailey, of the Connecticut Agricultural Experiment Station, New Haven, Conn.

In three of the samples the Fucoma was 0.675 per cent lower than the Mojonnier. Bringing the centrifuge up to speed again at the end of two minutes failed to eliminate the film but gave results which compared closely with the Mojonnier method. However, it is interesting to note that bringing the centrifuge up to speed a third time still further raised the test and almost eliminated the film. In every instance except one, the results after bringing the centrifuge up to speed a third time, were higher than the Mojonnier tests. The average of eleven tests of the same sample gave results 0.2 per cent higher than the Mojonnier. The importance and influence of length of centrifuging is also brought out in the summary of the two last columns in table 1. Thus bringing the centrifuge up to speed a second time at the end of two minutes raised the test 0.2 per cent, while a third bringing up to speed increased the test 0.5 per cent over centrifuging it for only four minutes.

In order to determine the maximum time required for centrifuging and to study the influence of a heated tester, a second series of samples were prepared. The steam turbine centrifuge was warmed up by turning on steam and allowing it to run idle for about ten minutes. This brought the centrifuge to a temperature of about 135° to 140°F. The samples were then placed in the heated tester and centrifuged for seven minutes with steam turned on during the entire time. After noting the nature of the fat column and the per cent of fat, the samples were centrifuged for two more minutes and read again. As is indicated in table 2, all fat columns were distinctly clear with no film. Whether the elimination of the film was due to the heated tester or centrifuging at constant speed for seven minutes is a question, but as there was still a slight film when the samples were centrifuged for 3 times two minutes, it seems reasonable to conclude that a heated tester aids materially in removing the film. The readings after seven minutes centrifuging checked closely with the Mojonnier, but an additional centrifuging of two minutes caused a higher reading in 4 out of 7 samples. Since results in table 2 indicated that centrifuging for seven minutes in a heated tester gave results which not only checked closely with the

Mojonnier, but fat columns which were free from the hazy film, we tried lowering the centrifuging time to six minutes with constant pressure and speed, and with steam turned on during the entire six minutes. Results reported in table 3 show that while the tests compare closely with the Mojonnier, six minutes centrifuging was not sufficient to entirely eliminate the hazy film in all of the fat columns. Therefore, after reading the samples, they were again placed in the centrifuge and whirled for another three minutes. This eliminated the film but also increased the size of the fat column. The data in table 3 further stresses the importance of length of centrifuging, but as shown in all the other tables also brings out that exhaustive centrifuging results in tests which are from 0.2 to 0.4 per cent higher than the Mojonnier. This probably is due to the use of amyl alcohol. A study of its chemical properties leads us to believe that some of the amyl alcohol mixes with the milk fat. The fat column therefore is not pure milk fat, but in reality a mixture of milk fat and amyl alcohol.

TABLE 2

*Influence of heating the tester and increasing the length of time of centrifuging on the clearness of fat column and final per cent of fat*

| MOJONNIER TEST | FUCOMA TEST RUN FOR | | | | | | INCREASE IN PER CENT OF FAT OVER FIRST FUCOMA READING | |
|---|---|---|---|---|---|---|---|---|
| | Seven minutes* | | Nine minutes* | | Nine minutes water bath† | | Nine minutes | Nine minutes plus water bath |
| C. A. C. ice cream mix, 10/20/24 14.48 to 14.45, average 14.465 | 14.6 | Fat column clear, no film | 14.7 | Fat column clear, no film | 14.8 | Fat column clear, no film | 0.1 | 0.2 |
| | 14.7 | | 14.9 | | 14.9 | | 0.2 | 0.2 |
| | 14.6 | | 14.6 | | 14.6 | | 0.0 | 0.0 |
| | 14.8 | | 14.8 | | 14.8 | | 0.0 | 0.0 |
| | 14.6 | | 14.8 | | 14.8 | | 0.2 | 0.2 |
| | 14.6 | | 14.6 | | 14.6 | | 0.0 | 0.0 |
| | 14.5 | | 14.6 | | 14.6 | | 0.1 | 0.1 |
| | AVERAGE VARIATION FROM MOJONNIER TEST | | | | | | AVERAGE VARIATION FROM FIRST FUCOMA READING | |
| | +0.163% | | +0.248% | | +0.265% | | 0.086% | 0.10% |

* Read from tester.

† Placed in water bath at 130°F. for three to four minutes.

However, it is also true that by carefully controlling the period of the centrifuging and the speed of the tester, results may be obtained which check closely with the Mojonnier. Thus when centrifuged for six minutes, 7 samples out of 10 checked within 0.15 per cent while the highest individual variation was 0.265 per cent. When centrifuged for seven minutes, 5 samples out of 7 checked within 0.15 per cent and the largest individual variation was 0.345 per cent. When it is recalled that considerable variations are encountered even on the Mojonnier tester, these results are surprisingly close on a product like ice cream.

TABLE 3

*Showing influence of centrifuging for six minutes with and without use of water bath*

<table>
<tr><th rowspan="2">MOJONNIER TEST</th><th colspan="6">FUCOMA TEST RUN FOR</th><th colspan="2">INCREASE OF PER CENT OF FAT OVER FIRST FUCOMA READING</th></tr>
<tr><th colspan="2">Six minutes*</th><th colspan="2">From water bath†</th><th colspan="2">Nine minutes‡</th><th>From water bath</th><th>Nine minutes</th></tr>
<tr><td rowspan="12">C. A. C. ice cream mix, 10/20/24 14.48 to 14.45, average 14.465</td><td>14.7</td><td rowspan="10">Film prominent</td><td>14.6</td><td rowspan="10">Slight film</td><td>14.8</td><td rowspan="10">No film</td><td>−0.1</td><td>+0.1</td></tr>
<tr><td>14.6</td><td>14.6</td><td>14.7</td><td>+0.0</td><td>+0.1</td></tr>
<tr><td>14.2</td><td>14.3</td><td>14.6</td><td>+0.1</td><td>+0.4</td></tr>
<tr><td>14.2</td><td>14.2</td><td>14.4</td><td>+0.1</td><td>+0.3</td></tr>
<tr><td>14.6</td><td>14.7</td><td>14.75</td><td>+0.1</td><td>+0.15</td></tr>
<tr><td>14.3</td><td>14.4</td><td>14.6</td><td>+0.1</td><td>+0.3</td></tr>
<tr><td>14.5</td><td>14.6</td><td>14.8</td><td>+0.1</td><td>+0.3</td></tr>
<tr><td>14.6</td><td>14.7</td><td>14.8</td><td>+0.1</td><td>+0.2</td></tr>
<tr><td>14.4</td><td>14.5</td><td>14.7</td><td>+0.1</td><td>+0.3</td></tr>
<tr><td>14.6</td><td>14.7</td><td>14.7</td><td>+0.1</td><td>+0.1</td></tr>
<tr><td colspan="6">VARIATION FROM AVERAGE MOJONNIER TEST</td><td colspan="2">VARIATION FROM FIRST FUCOMA READING</td></tr>
<tr><td colspan="2">+0.005%</td><td colspan="2">+0.065%</td><td colspan="2">+0.215%</td><td>0.090%</td><td>0.225%</td></tr>
</table>

In view of these results the authors suggest the following modifications from the Troy-Fucoma method:

1. Weigh out and prepare samples as per directions of the Fucoma Co., except that 6 cc. of water are used instead of 5 cc.

2. Place samples in heated centrifuge and bring the tester up to speed.

3. *Centrifuge for a period of not less than six minutes nor more than seven minutes at a constant and full speed.* This is perhaps best done in a steam turbine tester by regulating the steam pres-

TABLE 4

*Results of modified Fucoma method as compared with the Mojonnier method for determining the per cent of the fat in ice cream*

| ICE CREAM | MOJONNIER TEST | FUCOMA TEST | VARIATION FROM MOJONNIER |
|---|---|---|---|
| Chocolate | 9.46 | 9.6 | +0.14 |
| Chocolate | 9.48 | 9.65 | +0.17 |
| Chocolate | 12.26 | 12.30 | +0.04 |
| Chocolate | 12.19 | 12.10 | −0.09 |
| Strawberry | 7.98 | 8.10 | +0.12 |
| Strawberry | 7.95 | 8.05 | +0.10 |
| Vanilla | 9.87 | 9.80 | −0.07 |
| Vanilla | 9.69 | 9.80 | +0.11 |
| Vanilla | 11.62 | 11.70 | +0.08 |
| Vanilla | 11.61 | 11.60 | +0.01 |
| Vanilla | 12.89 | 13.00 | +0.11 |
| Vanilla | | 14.10 | −0.11 |
| | | 14.10 | −0.11 |
| | | 14.10 | −0.11 |
| | | 14.05 | −0.16 |
| C. A. C. vanilla ice cream mix | 14.20 to 14.22, average 14.21 | 14.20 | −0.01 |
| | | 14.10 | −0.11 |
| | | 14.15 | −0.06 |
| | | 14.20 | −0.01 |
| | | 14.10 | −0.11 |
| | | 14.20 | −0.01 |
| | | 14.15 | −0.06 |
| | | 14.00 | −0.21 |
| | | 14.30 | +0.09 |
| | | 14.40 | +0.19 |
| | | 14.00 | −0.21 |
| | | 14.20 | −0.01 |
| | | 14.40 | +0.19 |
| | | 14.30 | +0.09 |
| | | 14.40 | +0.19 |
| | | 14.30 | +0.09 |
| Chocolate ice cream mix | 8.775 to 8.793, average 8.78 | 8.70 | −0.08 |
| | | 8.80 | +0.02 |
| | | 8.80 | +0.02 |
| | | 8.70 | −0.08 |
| | | 8.75 | −0.03 |
| | | 8.80 | +0.02 |
| | | 8.75 | −0.03 |
| | | 8.90 | +0.12 |
| | | 9.00 | +0.22 |
| | | 8.90 | +0.12 |
| | | 9.00 | +0.22 |
| | | 9.00 | +0.22 |
| | | 9.00 | +0.22 |
| | | 9.00 | +0.22 |

All but 16 of the Fucoma tests reported in table 4 were conducted by George Brigham as partial fulfillment of the requirements of the course in Advanced Dairying.

sure so as to give the necessary revolutions per minute (1000 to 1200 revolutions per minute, depending on the diameter). The steam should be on during the entire run so as to maintain constant speed and also to maintain the heat in the tester. If no provision is made for heating the tester, it is a good plan to warm up the tester by allowing it to run empty with steam turned on for about 5 minutes just previous to centrifuging the samples.

4. After whirling the samples in a heated centrifuge for six to seven minutes, remove them from tester and place in a hot water bath at 130°F. for five minutes. Obviously the level of water should be such that the fat columns are immersed.

5. Read each sample directly from water bath.

Results on Fucoma tests made according to the suggested modifications are reported in table 4. This includes not only 25 tests of vanilla ice cream but 18 of chocolate and 2 of strawberry fruit ice cream.

A study of the data in table 4 shows that when the suggested modifications of Fucoma tests were followed:

22 samples or 48.88% of samples checked within 0.1% from Mojonnier
33 samples or 73.33% of samples checked within 0.15% from Mojonnier
38 samples or 84.44% of samples checked within 0.20% from Mojonnier
45 samples or 100.00% of samples checked within 0.25% from Mojonnier

These results check much closer with the Mojonnier than those reported in our first paper (1). This we believe is due to the careful standardization of: (1) the time and speed of centrifuging; (2) the temperature of centrifuging; (3) the temperature at which the fat columns were read.

## INFLUENCE OF TEMPERATURE OF FAT COLUMNS WHEN READ

There was some question as to the influence of the temperature of the water bath on the per cent of fat. To check this factor and to determine which temperature gave the closest comparable results with the Mojonnier, a series of tests were made in which samples were first read at a temperature of 140° to 135°F. and subsequently at 130° to 125°F. Results are reported in table 5. Practically all of the tests read at 140° to 135°F. gave results averaging about 0.1 per cent higher than those at 130° to 125°F.

As the results obtained at 130° to 125°F. checked closer with the Mojonnier, this temperature is suggested in preference to the higher temperature.

### ACCURACY OF THE TORSION BALANCE FOR WEIGHING THE CHARGE

A series of trials were run comparing the Torsion balance with the analytical balance. For this purpose a wire holder was devised for use in weighing charges with the Fucoma test bottles on the analytical balance. For comparison 24 tests were weighed out on the analytical balance and 24 on the Torsion cream balance. These total of 48 tests were then prepared and centrifuged in exactly the same manner. After centrifuging, all tests were read from a water bath at 130°F. Results are given in table 6. Judging from results in table 6, the Torsion balance is sufficiently accurate and compares favorably with the chemical balance.

TABLE 5

*Influence of varying temperature of the water bath on the per cent of fat obtained*

| MOJONNIER TEST | FUCOMA TESTS READ AT | | PER CENT OF VARIATION FROM SECOND OVER FIRST READING | VARIATION FROM MOJONNIER | |
|---|---|---|---|---|---|
| | 130 to 125°F. | 140 to 135°F. | | 130 to 125°F. | 140 to 135°F. |
| C. A. C. ice cream mix, 10/4/24<br>13.64 to 13.67, average 13.675 | 13.7 | 13.7 | +0.0 | 0.025 | 0.025 |
| | 13.8 | 13.9 | +0.1 | 0.125 | 0.225 |
| | 14.1 | 14.2 | +0.1 | 0.425 | 0.525 |
| | 13.9 | 13.9 | +0.0 | 0.225 | 0.225 |
| | 13.6 | 13.7 | +0.1 | 0.075 | 0.025 |
| | 13.8 | 13.9 | +0.1 | 0.125 | 0.225 |
| | 13.8 | 14.0 | +0.2 | 0.125 | 0.325 |
| | 13.6 | 13.6 | +0.0 | 0.075 | 0.075 |
| | 13.9 | 14.0 | +0.1 | 0.225 | 0.325 |
| | 13.7 | 13.8 | +0.1 | 0.025 | 0.125 |
| | 13.7 | 13.8 | +0.1 | 0.025 | 0.125 |
| | 13.8 | 13.9 | +0.1 | 0.125 | 0.225 |
| Average variation.................. | | | 0.083% | 0.125% | 0.204% |

### SUMMARY AND CONCLUSION

1. Further studies were made with the Troy-Fucoma method for determining the per cent of fat in ice cream. The object

was to determine the influence of (*a*) length of time of centrifuging (*b*) heating the centrifuge (*c*) and the temperature of the fat column when read. The Mojonnier method was used as a standard of comparison.

2. The accuracy of the Torsion cream balance for weighing the 5-gram charges used in the Fucoma method was determined by comparing results obtained with a Torsion balance with results when charges were weighed on an analytical balance.

TABLE 6

*Accuracy of Torsion balance when used for weighing 5-gram charge*

| MOJONNIER TEST | FUCOMA TEST | | VARIATION OF FUCOMA TESTS FROM MOJONNIER | |
|---|---|---|---|---|
| | Cream balance | Analytical balance | Cream balance | Analytical balance |
| C. A. C. ice cream mix, 10/4/24<br>13.64 to 13.71, average 13.675 | 13.4 | 13.6 | −0.275 | −0.075 |
| | 13.8 | 13.9 | +0.125 | +0.225 |
| | 13.9 | 14.1 | +0.225 | +0.425 |
| | 13.7 | 13.9 | +0.025 | +0.225 |
| | 13.2 | 14.0 | −0.475 | +0.325 |
| | 13.6 | 13.8 | −0.075 | +0.125 |
| | 13.7 | 13.8 | +0.025 | +0.125 |
| | 13.4 | 14.0 | −0.275 | +0.325 |
| | 13.8 | 14.1 | +0.125 | +0.425 |
| | 13.4 | 14.0 | −0.275 | +0.325 |
| | 13.4 | 14.0 | −0.275 | +0.325 |
| | 13.7 | 13.9 | +0.025 | +0.225 |
| | 13.7 | 13.9 | +0.025 | +0.225 |
| | 13.9 | 13.9 | +0.225 | +0.225 |
| | 14.2 | 14.2 | +0.525 | +0.545 |
| | 13.9 | 13.9 | +0.225 | +0.225 |
| | 13.7 | 14.1 | +0.025 | +0.425 |
| | 13.9 | 13.9 | +0.225 | +0.225 |
| | 14.0 | 14.0 | +0.325 | +0.325 |
| | 13.6 | 13.8 | −0.075 | +0.125 |
| | 14.0 | 14.0 | +0.325 | +0.325 |
| | 13.8 | 14.0 | +0.125 | +0.325 |
| | 13.8 | 13.9 | +0.125 | +0.225 |
| | 13.9 | 14.0 | +0.225 | +0.325 |
| Average variation........................... | | | 0.193% | 0.277% |

3. Centrifuging the ice cream samples for four minutes or 2 times two minutes in the Fucoma centrifuge gave variable results which in the majority of instances were lower than the

Mojonnier test. Fat columns frequently had a hazy film at the bottom of the fat column.

4. Centrifuging 3 times two minutes or six to seven minutes with constant speed gave results which checked closely with the Mojonnier test and fat columns which were clear and distinct.

5. Centrifuging for nine minutes with constant speed gave readings which were from 0.2 to 0.4 per cent higher than the Mojonnier test. High results are probably due to part of the amyl alcohol mixing with the milk fat and appearing in the fat column as a mixture of amyl alcohol and milk fat.

6. A heated tester greatly aided in eliminating the hazy film at the bottom of the fat column.

7. Readings compared closer to Mojonnier test when read at 130° to 125° than if a temperature of 140° to 135°F. is used for water bath.

8. A good Torsion balance is sufficiently accurate for use in weighing out charges for the Troy-Fucoma or Babcock method.

## REFERENCE

(1) FISHER, RICHARD C., AND WALTS, CHARLES C.: A comparative study of methods for determining the per cent of butterfat in dairy products. I. Ice cream. Journal of Dairy Science, Vol. viii, No. 1, January, 1925.

# THE DESTRUCTION OF BACTERIA IN THE ROLLER PROCESS OF MILK DRYING*

R. F. HUNWICKE AND H. JEPHCOTT

*From the Glaxo Research Laboratories of Messrs. Joseph Nathan & Co., Ltd.*

For several years past the dried milk prepared in the factories controlled by these laboratories has been subjected to daily bacteriological examination. The results of these examinations have demonstrated the remarkable degree of bacterial purity which can be attained by a careful attention to details of technique, and to the proper control of the conditions under which the machines are run.

Constant inoculation experiments have also demonstrated the complete freedom from tuberculous infection of dried milk prepared at these factoies. This might have been due to one of three causes:

*a.* Failure of the inoculated animals to react.
*b.* Freedom from tuberculous infection of the milk received at the source of manufacture.
*c.* Destruction during the drying process of B. tuberculosis, if present in the milk.

The probability of the first of these causes being responsible for the negative results is extremely remote when the large number of experiments is considered; it becomes even less likely when it is remembered that inoculations, by an identical technique, of animals with milk known to be infected with tuberculosis have produced positive results.

While the milk received at the factories is known to be in general free from tuberculous infection (this has on many occasions been demonstrated by actual inoculation experiments), there is, nevertheless, the possibility, where large volumes of milk are received, of slight tuberculous infection on some occasions.

While, therefore, (*b*) was a possible, if not a probable, cause

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* Received for publication December 8, 1924.

of the results obtained, it seemed desirable to investigate how far the drying process itself could be responsible for destruction of tuberculous infection in the event of its accidentally occurring in the milk supplies.

Moreover, ordinary routine bacteriological examinations are no guide as to the possibility of the survival of the pathogenic types, such as B. typhosus and pathogenic micrococci, which might conceivably be present in the original milk.

Apart from Delépine's work, there have been published, so far as the authors are aware, no experiments which place this matter beyond doubt.

Delépine (1) was concerned chiefly with B. tuberculosis; this was the only specific organism which he tested for viability after undergoing the drying of milk by the roller process. His observations on the survival of other types of bacteria in this process were confined to a general study of the flora of the milk before and after drying, and his conclusions were that while micrococci and non-sporing bacilli were not found in the dried milk as it came from the knives of the machine, sporing bacilli were fairly abundant. Delépine's observations on the bacillus tuberculosis will be considered in a later section of the present paper. Supplee and Ashbaugh (2) showed the extent of the reduction in the total bacterial content brought about by the roller process, and how this may be improved by care in technique at the factories. The same subject has been dealt with by the present authors (3).

None of these investigations, however, demonstrated the effect of the roller process upon various types of bacteria treated separately, and the present series of experiments was undertaken with this object.

## SCOPE OF THE INVESTIGATION

The types of bacteria to be investigated were four in number:

*a.* Non-sporing bacteria, including various types of micrococci
*b.* Sporing bacilli
*c.* B. tuberculosis (human)
*d.* B. tuberculosis (bovine)

Some living tubercle bacilli of bovine origin were found to have survived treatment (by the roller process).

Owing to the fact that a special technique is necessary in investigating B. tuberculosis, this part of the investigation is treated separately.

For the experiments with the other types of bacteria there were selected typical strains which may be taken as representative of the various main types into which bacteria can be grouped. No proteus strain was included, but apart from its strong proteolytic properties this organism is of the same general class as B. coli and B. cloacae, i.e., it is a small motile non-capsulated gram negative organism. It may be pointed out that the various strains tested, though not pathogenic strains, are all representative of pathogenic types, and evidence as to the destruction of B. coli is equally applicable to (for example) B. typhosus. A similar consideration applies to all the other strains tested.

The names of the organisms are set out in the tables, and there is nothing to be said of their characters, except that they were typical in each case.

### *1. Bacteria, other than B. tuberculosis*

*Experimental method.* Half a gallon of ordinary full cream milk was sterilized by keeping it just below 100°C. for one hour. A fresh agar slope culture of the organism to be tested was then emulsified into about 5 cc. of the cold milk and the emulsion added to the bulk when cold, which was allowed to stand overnight at room temperature.

A count of the bacteria in the milk before drying was made on agar plates kept for forty-eight hours at 37°C.

It is obviously undesirable that the drying of inoculated milk should be carried out in a factory and on machines engaged upon the production of dried milk for infant feeding. These experiments were, therefore, carried out upon the laboratory experimental drying plant. It may, however, be emphasized that this experimental drying plant is identical in every respect with the factory machines, with the exception that the length of the rollers is considerably reduced. The conditions maintained during these experiments, i.e., temperature of solutions, steam pressure, rate of flow of milk, etc., were identical with those ruling at all the factories; these conditions are determined by the laboratory and for them one of the present authors, who is thoroughly familiar with the factory conditions, is in part responsible. The milk receptacles, tanks and feeding pipes of the machine were sterilized before each experiment by means of live steam, and the walls of the drying room, which are lined with white tiles, were kept moist during the whole of the experiment.

The film of dried milk coming away from the knives of the machine was directed by means of sterile spatulae into sterile tins, the lids of which were replaced as soon as all the powder from each drying had been collected.

The dried milk was reconstituted by adding 7 parts of sterile water to 1 part of the powder, warming to 45°C. for ten minutes, and shaking well; 0.5 cc. of the reconstituted milk was then plated to nutrient agar in the usual manner. The plates were incubated for forty-eight hours at 37°C. before examination.

*Observations.* It will be seen from a study of table 1, that virtually a total destruction of the non-sporing types of bacteria took place. In only one case were there any survivors, and in that case the number of bacteria in the original milk was enormously greater than in any of the others. Moreover, a survival of four bacteria out of a total of 2,000,000,000 is quite negligible.

All pathogenic types likely to be met with in milk are non-sporers, and it is clear from the experiments cited that all of these will be totally destroyed in milk which is dried by the roller process. One organism, of unknown species, was a capsulated organism, having a well marked fat envelope. It might have been expected that a proportion of these would survive, but such was not the case, although as many as 320,000,000 per cc. were found in the milk before drying.

Turning now to table 2, it will be seen that two typical sporing saprophytes were tested, and that both showed a certain percentage of survivors after drying. This is to be expected, since the resistance of spores to heat is often remarkably great.

The survival of a certain number of sporing types is of no importance from the hygienic point of view, since no sporing strains which would be likely to be found in milk are pathogenic when administered orally. Nevertheless, the presence or absence of sporing bacteria in dried milk gives a valuable indication as to the purity or otherwise of the original milk used, especially as B. welchii (B. enteritidis sporogenes) is a particularly resistant spore-bearer of intestinal origin, whose value as an indicator of excretal contamination is well known. Moreover the presence of large numbers of ordinary aerobic sporing bacilli, which are readily detected by the characteristic appearance of their colonies on agar, would suggest that the general bacterial content of the original milk was high.

TABLE 1

*Non-sporing bacteria*

| LABORATORY NUMBER OF STRAIN | TYPE | NUMBER OF BACTERIA IN ORIGINAL MILK PER CUBIC CENTIMETER | NUMBER OF BACTERIA IN RECONSTITUTED DRIED MILK PER CUBIC CENTIMETER |
|---|---|---|---|
| A. 35 | B. coli | 300,000,000 | 0 |
| A. 6 | B. cloacoe | 2,000,000,000 | 4 |
| A. 57 | Capsulated, produces red pigment | 320,000,000 | 0 |
| A. 94 | Sarcina lutea | 1,000,000 | 0 |
| A. 95 | Staphylococcus albus | 510,000,000 | 0 |
| A. 97 | Streptococcus long chains | 10,000,000 | 0 |

TABLE 2

*Sporing bacteria*

| LABORATORY NUMBER OF STRAIN | TYPE | NUMBER OF BACTERIA IN ORIGINAL MILK PER CUBIC CENTIMETER | NUMBER OF BACTERIA IN RECONSTITUTED DRIED MILK PER CUBIC CENTIMETER |
|---|---|---|---|
| A. 11 | B. mycoides | 120,000,000 | 47,000 |
| A. 96 | B. subtilis | 2,000,000 | 50,000 |

### 2. *Bacillus tuberculosis*

Whether or no B. tuberculosis survives the roller process of milk drying is a matter of very great importance, in view of the great use which is now made of dried milk for infant feeding, and of the claim of the manufacturers that by its use all risk of infection by this organism is avoided.

In view of the results of Delépine's investigations (loc. cit.) it seemed advisable to confirm by direct experiments the negative results obtained from routine inoculations in these laboratories, and this section of the present investigation is the outcome.

*Review of Delépine's investigation.* Delépine in his experiments used 10 gallons of milk which contained 240 cc. of fresh tuberculous milk from a cow which had been under observation for about a month and was giving highly infected milk from three out of four quarters of the udder. In addition to this the 10 gallons of milk contained 250 cc. of milk cultures of the bovine tubercle bacillus, and 5 potato cultures of the same bacillus. Delépine demonstrated that each drop of the final mixture used in each experiment contained tubercle bacilli easily demonstrable. He considered that the number and virulence of the bacilli present in the treated milk were not in excess of what he had observed in the case of naturally infected milk.

He inoculated four guinea pigs with the original milk, two with milk from the churn just before the beginning of the experiment, and two with milk from the tank towards the end of the operations. Four guinea pigs were also inoculated with reconstituted milk taken (*a*) from one roller at the beginning of the operation, (*b*) from the other roller at the beginning of the operation, (*c*) from one roller towards the end of the operation, (*d*) from the other roller towards the end of the operation.

The results of the inoculation experiments are set out in great detail in Delépine's report, but it will only be necessary here to quote a portion of his summary:

> . . . . The tubercle bacilli which had survived . . . . were still capable of producing progressive tuberculosis in guinea pigs inoculated subcutaneously with milk containing these bacilli, but the

course of the disease produced by these bacilli was very much slower than that of the disease produced in guinea-pigs inoculated with the same amount of untreated tuberculous milk. The tuberculosis porduced by the heated bacilli was latent or occult for some four weeks.

Young rabbits fed with milk containing these modified bacilli did not contract tuberculosis.

## METHOD ADOPTED IN THE PRESENT INVESTIGATION

One gallon of fresh milk was taken, but not sterilized, since the presence of other bacteria was of no moment. This gallon was divided into two portions, one of which was inoculated with a strain of human B. tuberculosis, and the other with a strain of bovine B. tuberculosis, both strains having been obtained from the National Collection of Type Cultures, Lister Institute. The cultures were both grown on blood serum slopes. To inoculate the milk, about 5 cc. was added to the blood serum culture, and the growth completely scraped off into the milk. The 5 cc. of milk containing the tubercle bacilli was then added to the bulk of the milk in each case, and thoroughly mixed by stirring and shaking.

Fifty cubic centimeters of the milk were centrifugalized at 3000 r.p.m. for ten-minutes and the deposit mixed with 20 cc. of sterile water. Five cubic centimeters of this mixture were then injected into the left thigh of each of two guinea pigs with the usual aseptic precautions. Two guinea pigs were inoculated with the milk containing bovine tubercle bacilli, and two with that containing human tubercle bacilli.

The inoculated milks were dried on the machine already referred to, and under the same conditions as the previous experiments.

The dried powder was reconstituted with sterile water (1 in 8) and two guinea pigs were inoculated with the deposit from the sample which had contained human tubercle bacilli before drying, and two with that which had contained bovine tubercle bacilli.

The results of the inoculation experiments are given in table 3.

The presence of B. tuberculosis in the affected glands and organs was demonstrated by the Ziehl-Nielsen method of acid fast staining.

### OBSERVATIONS

The control animals show that both samples of milk were heavily infected with B. tuberculosis of human and bovine origin, respectively.

TABLE 3

| LABORATORY NUMBER | INOCULATED WITH | NUMBER OF WEEKS BEFORE POST MORTEM | RESULT OF POST-MORTEM |
|---|---|---|---|
| 107 | Milk containing human tuberculosis before drying | 8 | Tuberculous lesions of superficial and deep inguinal glands, liver and spleen |
| 108 | Milk containing human tuberculosis before drying | 9 | Tuberculous lesions of superficial and deep inguinal, popliteal and retrohepatic glands, and spleen |
| 109 | Milk containing bovine tuberculosis before drying | 6 (Died) | Tuberculous lesions of popliteal superficial and deep inguinal glands, liver and spleen |
| 110 | Milk containing bovine tuberculosis before drying | 9 | Tuberculous lesions of popliteal superficial and deep inguinal and retrohepatic glands and spleen |
| 111 | Milk containing human tuberculosis reconstituted after drying | 6 (Paralysis of left hind leg) | No lesions; all glands and organs normal. Well nourished healthy animal, except for paralysis of left hind leg |
| 112 | Milk containing human tuberculosis reconstituted after drying | 8 | Result same as above; perfectly healthy animal |
| 113 | Milk containing bovine tuberculosis reconstituted after drying | 9 | Result same as above |
| 114 | Milk containing bovine tuberculosis reconstituted after drying | 9 | Result same as above |

The other four animals which had been inoculated with the milk reconstituted from the dried milk prepared from these samples showed no lesions, and were all well nourished healthy animals.

The experiments show that the process of drying either killed the bacilli or rendered them avirulent, and confirm the claim of the manufacturers that dried milk prepared as in these experiments is perfectly free from tuberculous infection.

It will be seen that these results are not in agreement with those of Delépine referred to above. It is quite possible that a difference in the conditions of steam pressure and speed of the rollers account for the different results. As in the pasteurization of milk, so also in milk drying it is important that the conditions of heating both as regards time and temperature should be correct, and the present experiments show that under these conditions dried milk prepared from milk infected with B. tuberculosis is not capable of producing tuberculous lesions.

## SUMMARY

1. The roller process of milk drying is capable of destroying completely non-sporing bacteria.

2. The spores of spore bearing bacilli are not destroyed in all cases by the process; the number of survivors of this type of organism is probably dependent on the extent to which sporulation has occurred among them.

3. The roller process of milk drying is capable of either destroying or rendering completely avirulent B. tuberculosis of both human and bovine origin.

4. Dried milk adequately prepared by the roller process from highly tuberculous milk is incapable of causing tuberculous lesions in guinea pigs.

## REFERENCES

(1) Delépine: Report to L. G. B., Food Reports No. 21, 1914.
(2) Supplee and Ashbaugh: Jour. Dairy Sci., 1922, v, no. 2, 216.
(3) Hunwicke, R. F., and Jephcott, H.: Proc. Worlds Dairy Congress, 1923.

# INHERITANCE OF THE PER CENT OF FAT IN A HOLSTEIN HERD*

W. D. BURRINGTON[1] AND G. C. WHITE

*Dairy Husbandry Department, Storrs Agricultural Experiment Station, Storrs, Connecticut*

Very few results have been reported bearing upon the per cent of butter fat in the milk of the progeny of the female line. Most of the studies on this phase of butter fat inheritance have been developed around the male. The lack of emphasis on the female doubtless results from her relatively small number of progeny.

It is generally known that the milk of cows, even within a breed, varies considerably in the percentage of butter fat it contains. This variation is an individual characteristic. It has been quite definitely shown that each individual cow possesses a certain fairly definite capacity for the production of milk and butter fat, and that the milk, over a number of lactation periods, will contain a fairly constant per cent of fat reaching the correlation coefficient of 0.789 in Holsteins according to Gowen (1).

A study of the records of the Holstein herd at the Connecticut Agricultural College has revealed some interesting and valuable information concerning fat inheritance in two families. The fat percentage of the milk of the descendants of two cows, one testing 3.25 and the other 3.46 per cent, has remained apart to the fifth generation even though bulls were mated in common upon the two families.

## REVIEW OF LITERATURE

The mode of inheritance of economic functions in dairy cattle is at present little understood. The literature bearing upon the subject of fat inheritance is indeed scant, and little experimental work bearing directly on this particular problem has been done.

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*Received for publication February 6, 1925.

[1] The data were prepared and submitted in partial fulfillment of the requirements for the Master of Science Degree in 1924 by Mr. Burrington.

Recently several investigators have reported the results of studies on the inheritance of milk fat which suggest blended multiple factor inheritance, approaching a little nearer the lower testing parent. Notably among these is the work of Kildee and McCandlish (2), Olsen and Bigger (3), Castle (4), Ellinger (5), and Gowen (6). These results are based upon rather distinct crosses of pure breds, cross breds, and scrubs. Gowen (7) further states from an analysis of the Holstein Advanced Register that the sire exerts an influence upon his daughters (from different dams) expressed by the correlation coefficient of 0.5. Dams on their daughters by different sires have a correlation of 0.413 and he concludes that the sire and dam are about equal in their influence upon the offspring with reference to butter fat test. Furthermore, since the two have a combined correlation of about 0.5, it seems evident that they exert as much influence as all of the other ancestors combined.

## COLLECTION OF DATA

Two families of Holsteins have predominated the herd. These two families owed their foundation to two pure bred cows which were purchased in 1903. These cows were Fay M (35970) and Altoana Pietertje A (54656). When purchased, these cows were in calf to DeKol Burke (22991). Shortly after coming to the College herd, they each dropped a heifer calf. Subsequent gestations produced two more heifer calves. The original animals thus laid the foundation for the two families, and each of their daughters were progenitors of a strain within their respective families. Records are now available on five generations of Altoana Pietertje A descendants and four generations of Fay M descendants.

No females, other than the female descendants of these cows, were added to the herd during the greater part of the period under consideration. New blood was introduced into the herd from time to time by the purchase of bulls. These bulls were used indiscriminately on members of both families, although some bulls left daughters in only one family, due to very little use or to the small number of breeding females involved.

Since 1911, samples of milk from each member of the herd have been tested for butter fat at ten-day intervals. Upon the 10th, 20th, and 30th day of each month, samples were taken, both morning and evening, from each member of the herd. The single samples from each animal were composited in proportion to the yield of milk, and tested by the Babcock method. The milk was weighed and recorded after each milking. Earlier than 1911, determinations for fat were made monthly from composite samples. In many instances, the records used are those made under Advanced Registry regulations, being the average for a two-day period for each month.

The data presented here covers a period of twenty years, from 1903 up to and including part of 1923. The five generations of the Altoana Pietertje A family are represented by 16 animals which have completed 48 lactations. The 21 animals composing the four generations of the Fay M family have completed 53 lactations. In compiling the records of these animals, use is made only of lactation periods covering 200 days or more in length. The majority of the lactations cover periods from ten to fourteen months, although a few extend over a longer period. The longest period covers 943 days, while 14 cover less than 300 days. The number of lactations completed by the individual animal varies from one to eight; seven animals have five or more completed lactations, and four have only one.

The herd has been visited from time to time by its quota of disease, accidents, and defectives, although no wholesale disaster has hit it. Infectious abortion and its accompanying ravages have hampered the progress and production of the herd more than any other factor.

## PRESENTATION OF THE DATA

The summarized data are presented herewith in graph form.[2] The inherent testing capacity of the animal is determined by dividing the total fat yielded by the total milk.

Figure 1 shows clearly how the two families have tested through five and four generations respectively in relation to the breed average and the State legal standard for butter fat.

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[2] The complete data concerning each individual are being published in a Storrs (Conn.) Experiment Station bulletin.

The average per cent of fat for each generation was determined by taking the direct average of the cows represented. For example, if the first generation contained two animals, one testing 3.59 and the other 3.37, the average is taken to be 3.48 per cent $\left(\frac{3.59 + 3.37}{2} = 3.48\right)$

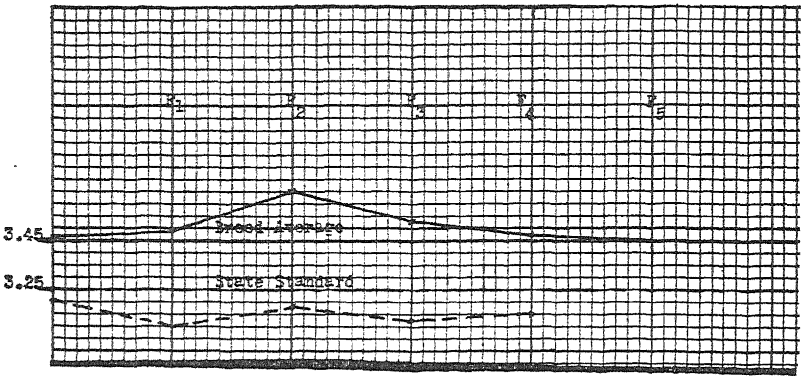

Fig. 1. Showing the Per Cent of Fat by Families and by Generations in Relation to Breed Average and Legal Standard

Solid line above represents the Altoana Pietertje A Family. Dotted line below represents the Fay M Family. Each space represents 0.05.

A study of this graph shows a striking difference in the average tests of these two families. The Altoana Pietertje A family has tested well above the legal standard for fat and has in no case fallen below the breed average of 3.45 per cent. The foundation cow of this family had a test of 3.46 per cent. The $F_1$, with two animals represented, averaged to test 3.48 per cent; the second generation, consisting of two animals, had an average test of 3.66 per cent; the next generation, composed of five animals, averaged to test 3.51 per cent; the three animals in the fourth generation had an average test of 3.47 per cent; and the fifth generation having only one animal and records available for only a partial lactation tested 3.46 per cent.

The original cow in the Fay M family averaged to test 3.25 per cent. This is 0.21 per cent lower than the foundation cow in the other family. This low testing trait has been passed on through four generations. The first generation progeny composed of two animals have an average test of 3.12 per cent; the second generation has seven animals which tested 3.23 per cent; the nine animals in the third generation have an average test of 3.17 per cent; and the one animal in the fourth generation having records available for only a partial lactation tested 3.2 per cent butter fat.

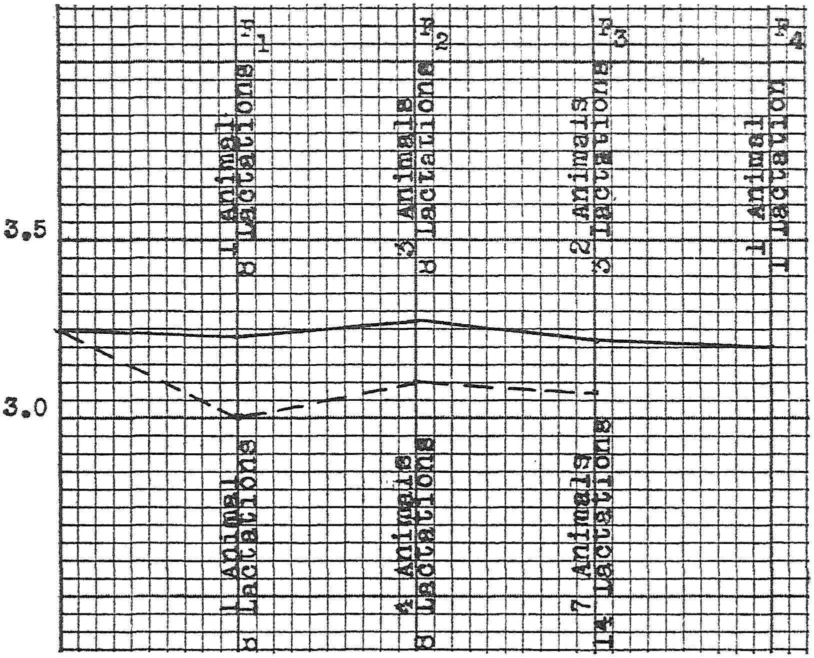

FIG. 2. SHOWING VARIATIONS IN THE PER CENT OF FAT BY STRAINS. FAY M FAMILY

Dotted line represents strain A. Solid line represents strain B. Each space represents 0.05.

It will be noted that the last generation represented in the higher testing family averaged to test identically the same as the original animal. The last generation represented in the other family tested slightly lower than the original animal.

There seems to be no tendency for the tests of these two families to come together. The curve representing the tests of the Altoana Pietertje A family is fairly straight except for the second generation, when it reached the peak with a test of 3.66 per cent, which is 0.2 per cent higher than that on either end of the curve. This is due to one very high testing cow, no. 202. The curve for the other family is more nearly a straight line, the original animal testing slightly higher than the average of any other generation of that family.

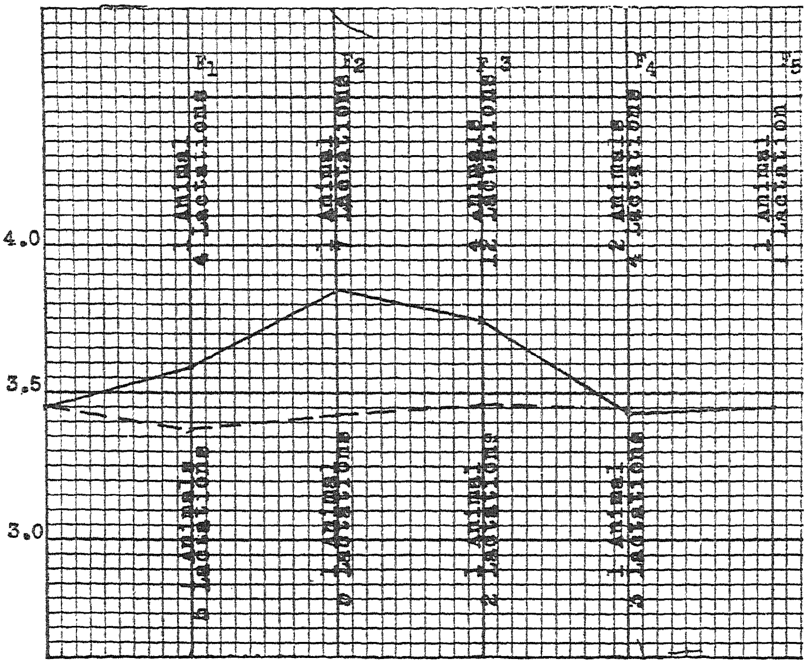

FIG. 3. SHOWING VARIATIONS IN THE PER CENT OF FAT BY STRAINS. (ALTOANA PIETERTJE A FAMILY

Solid line represents strain A. Dotted line represents strain B. Each space represents 0.05.

As shown in figure 2, strain B of the Fay M family has averaged to test considerably higher than strain A. The two cows in the first generation, those founding the separate strains, show a wider range in the per cent of fat than the animals in any other generation. The higher testing strain presents a much straighter line than does the lower testing group, and there appears to be a tendency for these two strains to come together. There is a noticeable decline in the per cent of fat for the higher testing strain and a positive, though not constant, tendency for the lower testing strain to increase in the per cent of fat.

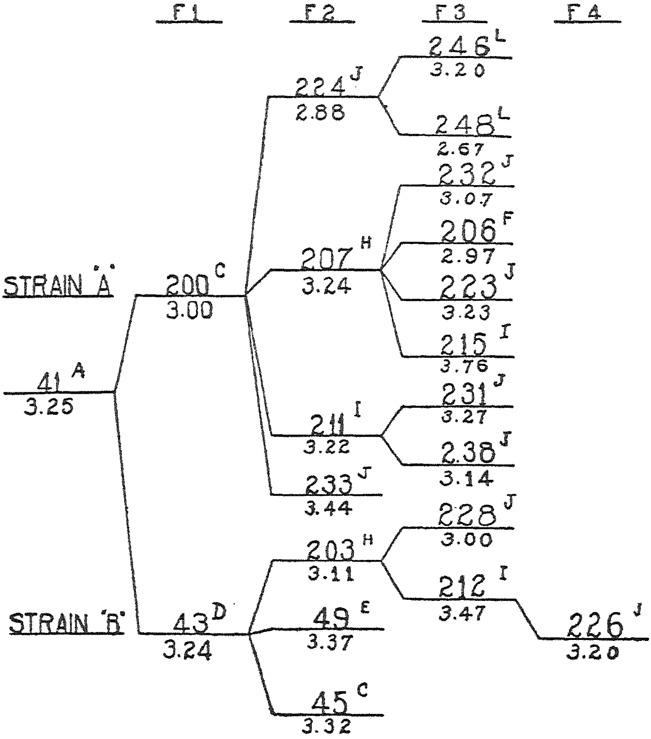

FIG. 4. FAY M FAMILY

Figure 3 shows that strain A of the Altoana Pietertje A family for the most part has continued to test considerably higher than strain B. The curves representing the two strains of this family follow somewhat the same trend as those of the Fay M family, although the variations are more pronounced. The extreme variation found in the second generation of the high testing strain can be attributed to the influence of one animal, no. 202, which was an unusually high tester for the breed. The third generation shows a slightly greater variation than the first. The two strains converge in the fourth generation. The per cent of fat of strain A for the fourth and fifth generations has descended to the average of that of the cow which founded these two strains. Records are not available for the fifth generation of the lower testing strain.

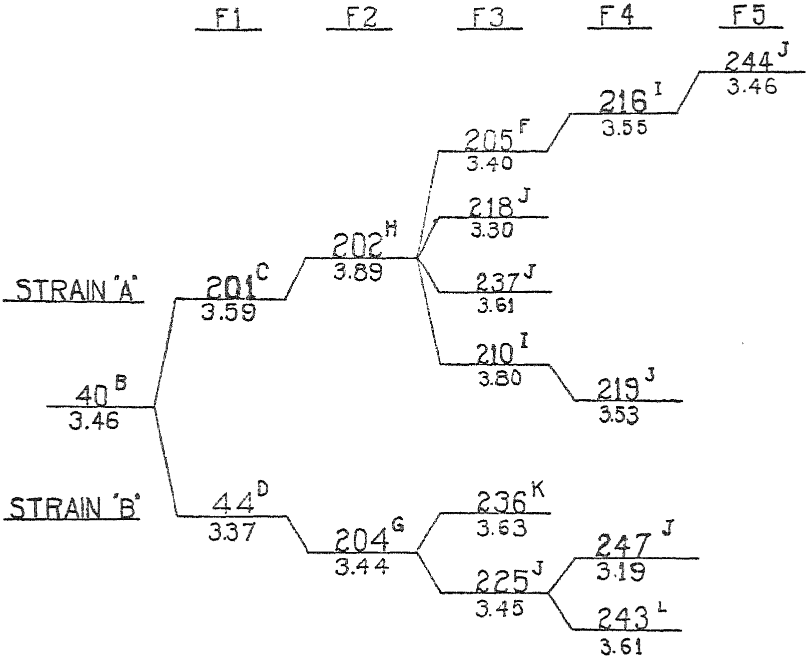

FIG. 5. ALTOANA PIETERTJE A FAMILY

Data on the individual animals are shown in more detail in figures 4 and 5 in which the number of the animal is given together with its average fat test. The diagram arrangement indicates the dam and the progeny of each and the letter identifies the sires.

Interesting facts brought out in these figures are that only two daughters in the low testing family, viz.: Nos. 212 (3.47 per cent) and 215 (3.76 per cent) have reached the original test of the high test family. These two daughters are by a son of a cow in the high test family. Further, there is only one cow in the high test family that has tested below the foundational test of the low test family, namely, no. 247 (3.19 per cent).

TABLE 1

| BULLS USED | ALTOANA PIETERTJE A FAMILY (HIGH TEST) | | | | | | | FAY M FAMILY (LOW TEST) | | | | | | | DIFFERENCE IN PER CENT OF FAT IN FAVOR OF HIGH TESTING FAMILY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High testing strain | | | Low testing strain | | | Average per cent of fat for both strains | High testing strain | | | Low testing strain | | | Average per cent of fat for both strains | |
| | Number of daughters | Number of lactations | Average per cent of fat | Number of daughters | Number of lactations | Average per cent of fat | | Number of daughters | Number of lactations | Average per cent of fat | Number of daughters | Number of lactations | Average per cent of fat | | |
| C | 1 | 4 | 3.59 | | | | 3.59 | 1 | 2 | 3.2 | 1 | 8 | 3.00 | 3.10 | 0.53 |
| D | | | | 1 | 5 | 3.37 | 3.37 | 1 | 1 | 3.24 | | | | 3.24 | 0.13 |
| E* | | | | | | | | 1 | 1 | 3.37 | | | | 3.37 | |
| F | 1 | 3 | 3.40 | | | | 3.40 | | | | 1 | 4 | 2.97 | 2.97 | 0.43 |
| G* | | | | 1 | 5 | 3.44 | 3.44 | | | | | | | | |
| H | 1 | 7 | 3.89 | | | | 3.89 | 1 | 5 | 3.32 | 1 | 6 | 3.24 | 3.28 | 0.61 |
| I | 2 | 5 | 3.67 | | | | 3.67 | 1 | 1 | 3.47 | 2 | 4 | 3.49 | 3.48 | 0.19 |
| J | 4 | 9 | 3.47 | 1 | 1 | 3.19 | 3.33 | 2 | 3 | 3.10 | 5 | 11 | 3.14 | 3.12 | 0.21 |
| K* | | | | 1 | 2 | 3.45 | 3.45 | | | | | | | | |
| L | | | | 1 | 2 | 3.66 | 3.61 | | | | 2 | 3 | 2.93 | 2.93 | 0.68 |

* Bulls K, G and E are of no significance here because each has only one daughter and no comparisons can be made. The records are supplied simply for completeness.

## INFLUENCE OF THE BULLS

Table 1 was constructed to determine what influence the several bulls used in the herd may have had on the per cent of fat. A summary of the daughters of each bull is given by strains and by families. Comparisons are made for the daughters of each bull in both strains of each family. A summary of the difference between the families is given in the right hand column.

Naturally variations are found in daughters of the same bull within the same female line. This variation also has considerable range between the families, varying from 0.68 per cent for bull L (the upper limit) to 0.13 per cent for bull D (the lower limit).

Three of the bulls have daughters in only one strain of one family. However, the seven bulls with daughters in both families give conclusive evidence that the female influence in determining the percentage of fat in the milk of their progeny in this herd was overpowering.

It will be noted that in every case animals by the same sire in the Altoana Pietertje A family have averaged to test above those in the Fay M family. Some bulls that did maintain the average high test in the Altoana Pietertje A family had difficulty in raising the low average of the low testing family, and in a number of cases the test was lowered. This would indicate that there is a tendency for the progeny to resemble the low testing parent. These results are similar to those obtained by Gowen in his study of cross bred daughters of a dairy-beef cross. It further illustrates that the prepotency of the low testing family for low fat tests could not be overcome by the bulls that could maintain an average high test in the other family. Thus it seems that the female may exhibit prepotency for such characters to a high degree.

*Reference to bulls used in the herd*

A. Count Pietertje Beets 16618
B. Sir Pietertje Lamb 21391
C. Hubbard Netherland DeKol 35221
D. DeKol Burke 22991
E. Crown Prince DeKol Paul 30569
F. Sir Hubbard Pietertje 48557 (sire is C; dam is no. 40)
G. Sir Inka DeKol Lilith 35261
H. Minnie Hark Prince Korndyke 41660
I. Minnie Hark Pietertje Burke 57476 (sire is H; dam is no. 44)
J. King Segis Inka Fayne 97699
K. Segis Fayne DeKol Pietertje 165985 (sire is J; dam is no. 201)
L. King Korndyke Fannie Veeman 199675.

It is unfortunate from the standpoint of such a study that so many different bulls were employed and so few daughters resulted from their use. It should be remembered, however, that such a study was not contemplated and that the conditions are a natural consequence of maintaining a small herd over a period of twenty years. Some of the bulls did not belong to the College herd.

No attempt has been made to evaluate the fat inherited propensities of the bulls themselves, since short time seven-day tests enter so largely into their pedigree records. However, some of the bulls used are from dams of the high testing family, viz.: Bull F is from the foundation cow 40; bull I is from cow 44, strain B; and bull K is from cow 201, strain A. Table 2 will permit a consideration of such records as are available of these bulls.

In table 2 is given the brief summary of the bulls that were used in the herd that are progeny of certain cows in the high testing strain. Because of the relatively few instances they are not dealt with by genetical precision. The test of the dams of these bulls is given as is also their daughters. Furthermore, the tests of the daughters of the sires of these "home bred" bulls is given. The small numbers in parenthesis indicate the number of animals involved in each case.

TABLE 2

*Records of bulls bred from herd*

| BULL | DAM | DAM'S DAUGHTERS | BULLS' OWN DAUGHTERS | | BULL'S SIRES' DAUGHTERS | |
|---|---|---|---|---|---|---|
| | | | High strain | Low strain | High strain | Low strain |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| F | 3.46 | 3.48 (2) | 3.40 (1) | 2.97 (1) | 3.59 (1) | 3.05 (2) |
| I | 3.37 | 3.44 (1) | 3.58 (2) | 3.48 (3) | 3.89 (1) | 3.28 (2) |
| K | 3.59 | 3.89 (1) | 3.45 (1) | | 3.48 (6) | 3.19 (7) |

Bull F is from the foundation cow 40, whose test was 3.46 per cent. Bull I is from no. 40's daughter, 44, the progenitor of strain B, and bull K is from no. 40's other daughter, 201, progenitor of strain A. The average of no. 40's daughters is 3.48 per cent. Bull F, her son, sired a daughter in each of the families testing 3.40 and 2.97 per cent respectively. The sire of bull F had daughters in both families testing 3.59 and 3.05 per cent, respectively. He thus lowered the test of his daughters under his dam and her daughters as well as his sire's daughters.

By the same manner of comparison, it is seen that bull I from the strain B cow (test 3.37) produced daughters in the two families that tested 3.58 and 3.48 respectively. Judging from one daughter of his sire (3.89 per cent), bull I might have received a factor of high test from his sire. However, two other daughters of his sire in the low family test only 3.28, which evidence does not mark him as prepotent for high test as these two daughters' tests were 3.24 and 3.32 per cent. Bull I did possess, evidently, by virtue of the dam's line the propensity for transmitting high tests. He is sire of the two highest testing cows in the low test family, viz.: No. 215 (3.76 per cent) and no. 212 (3.47 per cent). His third daughter, no. 211, in the low testing family tested 3.22 per cent. The first two are the only cases where the low test family crossed the average test line of the high test family.

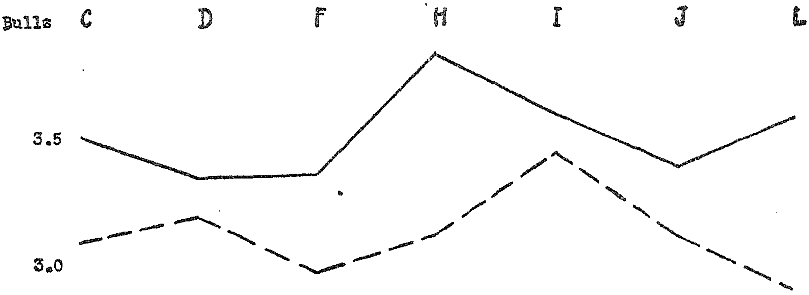

FIG. 6. SHOWING THE PER CENT OF FAT IN BOTH FAMILIES BY DAUGHTERS OF THE SEVERAL BULLS

Solid line represents the average per cent of fat in Altoana Pietertje A family of the daughters of the several bulls. Dotted line represents the average per cent of fat in the Fay M Family of the daughters of the several bulls. Each space represents 0.05.

Bull K having only one daughter does not throw any light on the subject. Although his dam, no. 201, produced one daughter having a test of 3.89 per cent, his (K) own daughter tested 3.45 per cent.

Figure 6 presents the average test of the seven bulls that have produced daughters in both families. They are given in the order of their use in the herd, the comparisons being made with respect to the relation to the original cows. In the case of C and D they were used in the herd during the same period of time. Since the daughters of these bulls may be and usually are products of more than one generation from the females, a progressive curve cannot be established. Compared to the original cow C raised the high and lowered the low test families a trifle; D lowered the high test and maintained the low test line; F lowered both families; H raised both; I raised both families; J lowered both lines; and L raised the high test slightly and lowered the low test considerably. Thus the bulls have apparently registered their own influence.

Thus with the limited data it is apparent that the bulls have influenced the test of the daughters but they have failed utterly to bring the two families together.

These results are somewhat contrary to the general belief and to genetically established facts. It is generally considered among dairy cattle breeders that the male is prepotent over the females to which he is bred. This is often the case in actual practise so far as milk yield is concerned, because the sire is usually the better bred animal. But the trite expression that "the bull is half of the herd" arises from the fact that the male has a numerical, rather than a biological advantage.

## PRACTICAL CONSIDERATIONS

It has not been necessary to sacrifice on milk yield in order to maintain a comparatively high butter fat test, as is often assumed. The high testing family produced an average of 11,402.6 pounds of milk per lactation (not exceeding 365 days of which 14 of the 48 are by Advanced Registry) with an average test of 3.54 per cent of fat, while the low testing family produced 11,274.85 pounds of milk which tested 3.12 per cent, of which 16 of the 53 lactations are by Advanced Registry test. The difference in the milk yield of these two groups was negligible (275.8 pounds), but the average yearly fat production differed by 53 pounds.

The summary of the production records shows clearly the difference in the food value of the milk produced by the two families. The high testing family produced milk which averaged 12.47 per cent total solids, and the low testing family averaged 11.29 per cent. This would indicate that the milk produced by the high testing family contained 9.46 per cent more food value per unit.

Too great emphasis cannot be put upon the importance of foundation animals. Of course, it is not possible to know for a certainty how good an individual will prove to be as foundation stock, but every means of selection known, and especially the buying of cows out of families that have proved their ability to produce and transmit desirable qualities to their progeny should be used.

## SUMMARY

In 1903 two Holstein cows were purchased. One of them had a butter fat test of 3.46 per cent and the other tested 3.25 per cent. These cows each produced two daughters, each of which formed a strain in the herd.

Altogether, Altoana Pietertje A (3.46 per cent) has produced 15 progeny that have milked through one or more 200 day lactation periods in the herd; and Fay M (3.25 per cent) has produced 20 progeny with similar performance. Represented here are six generations of Altoana Pietertje A and five generations of Fay M.

Even though sires have been used in common upon these two families, the tests remain as far apart today as originally. Only one in the high testing family has tested less than 3.25 per cent and only two in the low testing family (daughters of a son of a high test family cow) have tested above 3.46 per cent. The high test family has reached as high as 3.89 per cent and the low test family as low as 2.67 per cent in individuals.

Averaged by generations, the high test family maintains a test equal to the average for the breed, while the low test family usually is below the legal standard for fat. Low fat test in this family has behaved in dominant fashion. The one case of a

high test in the low test family (3.76) being from a daughter of a son from the high test family seems to be a case of Mendelian inheritance.

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# EFFECT OF INGREDIENTS IN THE ICE CREAM MIX ON ITS FREEZING POINT*

E. H. PARFITT AND C. L. TAYLOR[1]

*Dairy Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana*

The freezing of the ice cream mix is one of the important steps in the manufacture of ice cream and while some work has been done on this subject, there has been no detailed study in which all influencing factors have been involved and the actual freezing points determined. In commercial freezing of the ice cream mix there is found a wide variation of brine temperatures and therefore a detailed study regarding the effect of ingredients on the freezing point of the ice cream mix will be of timely interest and value to the ice cream industry.

Baer (1) says that the time of freezing is affected only slightly by the amount of sucrose (cane sugar) in the mix. He explains that sucrose is in true solution in the mix and that the amount of sugar in the mix is directly proportional to length of time required to freeze. He found that the ice cream mix usually froze at 31°F.

Mojonnier and Troy (2) state that the greatest part of the depression in the freezing point of the ice cream mix is caused by the milk and cane sugars.

Bowen and Thomas (3) found that the freezing point of milk depended upon its composition.

The object of this experiment was to determine the effect the following ingredients have upon the freezing point of the ice cream mix: milk fat, sucrose, lactose, casein and albumen, gelatin, the production of acid, ageing and neutralizers.

The mixes were made up and frozen in a Hortvet Cryoscope which was equipped with a Centigrade thermometer graduated in degrees from 30 above, to 30 below zero, so that with the aid of

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*Received for publication February 24, 1925.

[1] Student assistant.

a magnifying glass 1/10 of a degree could be read accurately. This thermometer had been checked with distilled water and definite percentage solutions of sucrose and was found accurate with 0.1°C. The freezing point was taken as the point at which the thermometer remained constant after there had been a rapid rise, due to the absorption of latent heat. The directions for use of the Hortvet Cryoscope as given by Emier and Amend (4) were followed. Duplicate determinations were made in all trials, and where there was variation in duplicate a triplicate determination made.

## METHOD OF PROCEDURE

A standard mix was made up using the composition shown in table 1.

The skimmilk powder was dissolved in the cream and skimmilk and heated to 145°F. on a water bath, in which the surrounding water did not exceed 155°F. The gelatin and sugar mixture was then added and the entire mix held for twenty minutes at 145°F. and then cooled immediately.

A sample of this mix was tested on the Mojonnier tester for milk fat and total solids. The method of procedure on these tests was the same as outlined in the "Technical Control of Dairy Products" by Mojonnier and Troy (2). The sample was also tested for acidity, using Mann's Acid Test, as described in "Modern Methods of Testing Milk and Milk Products" by L. L. Van Slyke (5).

This standard mix was altered by change of various ingredients, but in all cases was balanced so as to contain the same proportion of ingredients except that ingredient which was under observation.

Table 2 shows that when the fat content is varied from 8 to 17 per cent, the freezing point remains constant. It appears from these results, that milk fat has no effect upon the freezing point of the ice cream mix. However, it must be considered that by adding fat or cream to a mix the sucrose content is reduced, but the lactose content will increase.

In this experiment the milk fat was increased by adding pure milk fat and the sucrose content made up so that the percentage concentration of sucrose remained the same. There is a slight reduction of the lactose concentration in the mix but so slight as to come within the experimental error of the thermometer.

TABLE 1

| INGREDIENT | AMOUNT OF INGREDIENT | PER CENT SERUM SOLIDS | PER CENT TOTAL SOLIDS | PER CENT MILK FAT |
|---|---|---|---|---|
| | *grams* | | | |
| Cane sugar | 120 | | 11.4 | |
| Gelatin | 5 | | 0.47 | |
| Skimmilk powder | 60 | 5.7 | 5.7 | |
| 35 per cent cream | 228 | 1.1 | 9.3 | |
| Skimmilk | 587 | 5.4 | 5.4 | 7.98 |
| | 1000 | 12.2 | 32.27 | 7.98 |

Lactose content = 6.2 per cent. Acidity = 0.28 per cent. Freezing point = −2.0°C.

TABLE 2

*Effect of milk fat on freezing point of the standard ice cream mix*

| FREEZING POINT | PER CENT SUCROSE | PER CENT TOTAL SOLIDS | PER CENT SERUM SOLIDS | PER CENT MILK FAT |
|---|---|---|---|---|
| *°C.* | | | | |
| −2.0 | 12.0 | 33.4 | 12.2 | 8.3 |
| −2.0 | 12.0 | 39.7 | 10.9 | 17.6 |

TABLE 3

*Effect of sucrose on freezing point of the standard ice cream mix*

| FREEZING POINT | PER CENT SUCROSE | PER CENT TOTAL SOLIDS | PER CENT SERUM SOLIDS | PER CENT FAT |
|---|---|---|---|---|
| *°C.* | | | | |
| −2.0 | 12.0 | 33.4 | 12.2 | 8.3 |
| −2.4 | 14.3 | 35.1 | 11.8 | 8.0 |
| −2.7 | 15.8 | 36.3 | 11.6 | 7.9 |
| −3.0 | 17.5 | 36.4 | 11.3 | 7.7 |
| −3.6 | 19.2 | 39.2 | 11.1 | 7.5 |

The effect of sucrose on the freezing point is to decrease the freezing point. This decrease takes the form of a regular curve and the variation caused by 1 per cent is practically constant.

The other factors (lactose, milk fat, casein, lactic acid) in the mix were changed to such a slight degree, by the addition of sucrose, that they may be regarded as causing no change on the freezing point within 0.1°C.

TABLE 4

*Effect of lactose on freezing point of the standard ice cream mix*

| FREEZING POINT | PER CENT LACTOSE | PER CENT TOTAL SOLIDS | PER CENT SERUM SOLIDS | PER CENT FAT |
|---|---|---|---|---|
| *°C.* | | | | |
| −2.0 | 6.2 | 33.4 | 12.2 | 8.3 |
| −2.2 | 9.2 | 35.4 | 14.8 | 8.0 |
| −2.4 | 10.9 | 36.8 | 16.6 | 7.9 |
| −2.7 | 12.7 | 37.1 | 18.3 | 7.7 |
| −3.2 | 15.6 | 40.1 | 21.0 | 7.4 |

TABLE 5

*Effect of age on freezing point of the standard ice cream mix*

| FREEZING POINT | TIME (AGE) | PER CENT TOTAL SOLIDS | PER CENT SERUM SOLIDS | PER CENT FAT |
|---|---|---|---|---|
| *°C.* | | | | |
| −2.0 | None | 33.4 | 12.2 | 8.3 |
| −2.0 | 3 days | 33.4 | 12.2 | 8.3 |
| −2.0 | 1 week | 33.4 | 12.2 | 8.3 |
| −2.0 | 2 weeks | 33.4 | 12.2 | 8.3 |
| −2.0 | 4 weeks | 33.4 | 12.2 | 8.3 |

TABLE 6

*Effect of egg albumen on freezing point of the standard ice cream mix*

| FREEZING POINT | PER CENT CASEIN | PER CENT TOTAL SOLIDS | PER CENT SERUM SOLIDS | PER CENT FAT |
|---|---|---|---|---|
| *°C.* | | | | |
| −2.1 | 5.6 | 33.0 | 12.0 | 8.1 |
| −2.3 | 8.4 | 35.0 | 14.6 | 7.8 |
| −2.5 | 10.3 | 36.3 | 17.4 | 7.7 |
| −2.6 | 12.2 | 37.7 | 18.3 | 7.5 |
| −2.9 | 15.0 | 39.0 | 20.8 | 7.3 |

The results obtained from the effect of lactose on the freezing point of a standard ice cream mix shows that an increase in the lactose content lowers the freezing point of the standard ice cream mix, provided the sucrose content is maintained at the original percentage.

It also shows that as the percentage of lactose is increased the variation caused by 1 per cent of lactose decreases and the curve obtained from the variation in the freezing point caused by lactose practically coincides with that made by sucrose. In the work on lactose, it was also found that when there was a large percentage of lactose in the mix the lactose would tend to crystalize out of solution before the freezing point was reached. This difficulty was overcome by having the ether at a fairly low temperature, thus inducing rapid freezing.

The results obtained from the effect of age on the freezing point of the ice cream mix shows that there was no variation in the freezing point. The acidity of the mix at start with 0.28 per cent and in 4 weeks 0.30 per cent. The mix was kept at a temperature between 33 and 34°F.

Table 6 shows that an increase in the egg albumen content (used as a substitute for casein) will produce a lowering of the freezing point. However, the effect is not so apparent as is the effect of sucrose and lactose.

In the work on sucrose, when the altered mix had a sucrose content of 19 per cent and a lactose content of 5.5 per cent, considering the water content only, the combined percentage of these two ingredients equals 39 per cent of the water content. This combined percentage lowered the freezing point of altered mix to −3.6°C. The sucrose and lactose content of the altered mix containing egg albumen was 17.5 per cent. This on a water content basis is 29.4 per cent of the water content.

Therefore, calculating on a water content basis it is safe to conclude that if a 39 per cent solution of sucrose and lactose lowered the freezing point to −3.6°C., then 29.4 per cent solution should lower it to −2.8°C. In the actual results the altered mix containing egg albumen froze at −2.9°C. The explanation of the lowering of the freezing point by a high concentration egg albumen is that the water content in the mix is reduced, making the sugar content more concentrated. By doing this, a lowering of the freezing point is brought about.

Plummer (6) states that colloidal solutions have a low osmotic pressure, and that the freezing point of the continuous phase is lowered very slightly and the boiling point is raised very slightly.

Gelatin which has had its jelling strength destroyed when added to a mix up to 4.1 per cent will not cause a variation in the freezing point. Gelatin when it makes up more than 2.5 per cent of a mix, will cause the mix to become highly viscous. This high viscosity will cause the temperature of the mix to be lowered so slowly that the point of latent heat absorption is not detectable. The only method by which the freezing point can be determined is to watch for the formation of small ice crystals which indicates that the mix is frozen. This shows very nicely that colloids have but slight effect on the freezing point which is well within the error of this work.

TABLE 7

*Effect of gelatin on the freezing point of the standard ice cream mix*

| FREEZING POINT | PER CENT GELATIN | PER CENT TOTAL SOLIDS | PER CENT SERUM SOLIDS | PER CENT FAT |
|---|---|---|---|---|
| °C. | | | | |
| −2.1 | 0.5 | 33.0 | 12.0 | 8.1 |
| −2.1 | 1.1 | 32.7 | 11.6 | 7.8 |
| −2.1 | 1.7 | 32.4 | 11.4 | 7.7 |
| −2.1 | 2.9 | 32.0 | 10.8 | 7.3 |
| −2.1 | 4.1 | 31.6 | 10.2 | 6.9 |

TABLE 8

*Effect of souring on the freezing point of the standard ice cream mix*

| FREEZING POINT | PER CENT AS LACTIC ACID | PER CENT TOTAL SOLIDS | PER CENT SERUM SOLIDS | PER CENT FAT |
|---|---|---|---|---|
| °C. | | | | |
| −1.8 | 2.3 | 32.3 | 12.0 | 8.0 |
| −2.0 | 2.8 | 32.3 | 12.0 | 8.0 |
| −2.1 | 3.4 | 32.3 | 12.0 | 8.0 |
| −2.3 | 8.2 | 32.3 | 12.0 | 8.0 |
| −2.4 | 8.5 | 32.3 | 12.0 | 8.0 |

Results obtained on the effect of souring on the freezing point of the ice cream mix shows that souring lowers the freezing point of the mix, but that the lowering is not regular. It can be seen that the variation due to 1 per cent increase in lactic acid is very regular, decreasing as the per cent of acid increases. The work on the effect of lactic acid was not extensive enough to point out any special reason for the irregularity in the lowering of the freezing point.

Results obtained in this work show that a small quantity of C.P. sodium bi-carbonate will lower the freezing point; 1.3 grams of sodium bicarbonate added to 100 grams of mix lowered the freezing point 0.5°C.

TABLE 9

*Effect of neutralizer on the freezing point of the ice cream mix*

| FREEZING POINT | PER CENT LACTIC ACID | PER CENT TOTAL SOLIDS | PER CENT SERUM SOLIDS | PER CENT FAT |
|---|---|---|---|---|
| °*C.* | | | | |
| −2.4 | 3.4 | 34.3 | 12.4 | 8.7 |
| −2.9 | 2.0 | 34.3 | 12.4 | 8.7 |

## SUMMARY

1. Milk fat has no effect on the freezing point of the ice cream mix.
2. Increasing the sucrose content of the ice cream mix will lower the freezing point in direct proportion to the amount of sucrose added to the mix.
3. As a percentage of sucrose is increased the variation caused by 1 per cent is constant.
4. The effect of increasing the lactose content of the ice cream mix is that of lowering the freezing point with the same regularity that the freezing point is lowered by sucrose.
5. The variation caused by 1 per cent of lactose steadily decreases as the total percentage of lactose in the mix increases.
6. Egg albumen (casein) does not affect the freezing point of the ice cream mix.
7. Gelatin that has had its jelling strength destroyed by heating does not affect the freezing point of the ice cream mix.
8. Souring lowered the freezing point of the ice cream mix but the lowering is not regular.

9. Neutralizer in the form of sodium bi-carbonate when added to the ice cream mix caused a lowering of the freezing point.

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# SOY BEAN OILMEAL AND GROUND SOY BEANS AS PROTEIN SUPPLEMENTS IN DAIRY RATIONS*

L. H. FAIRCHILD AND J. W. WILBUR

*Department of Dairy Husbandry, Purdue University, Lafayette, Indiana*

It has been a problem to develop on Indiana farms crops which would supply sufficient protein in the dairy ration. Up to this time cottonseed meal, linseed oilmeal and gluten meal have been the chief high protein concentrates that have been used in the dairy ration. As all of these feeds must be purchased and brought to the farm, a much smaller quantity of them has been fed than that which should be fed.

Recently there has been considerable interest in the growing of soy beans on Indiana farms, and inquiries have been very frequently made by Indiana dairymen as to whether soy beans could be used as protein supplements in the dairy ration.

A number of early feeding trials have been made comparing soy bean oilmeal and soy bean cake with cottonseed meal as a protein supplement in the dairy ration, and they were found equal to or superior to cottonseed meal for production. Similar results have been obtained with ground soy beans (1) (2) (3). At the Indiana station (4), it has been found that ground soy beans together with a mineral mixture might be supplemented with good results for purchased protein concentrates in the ration of hogs. Feeding trials conducted recently (5) indicate that ground soy beans can be supplemented for linseed oilmeal in the dairy ration, and that soy bean oilmeal has practically the same feeding value as linseed oilmeal for milk production.

It has been suggested by some feed manufacturers interested in soy bean products, that farmers might send their soy beans to the mills where the oil would be extracted and the oilmeal returned to the farmers, the miller accepting the oil for his part of the transaction.

In the feeding of ground soy beans, the preparation of this feed

*Received for publication January 10, 1925.

can be done entirely on the farm, as these beans can be ground or crushed for the grain mixture. Some difficulty has been reported in regard to grinding soy beans because of their oil content, but the addition of some oats to the soy beans during the grinding process practically eliminates this difficulty.

An experiment, divided into two parts, has recently been completed at the Purdue Experiment Station. The first part of this experiment was conducted to compare the value of soy bean oilmeal with linseed oilmeal as protein supplements in the grain ration of the dairy cow. The second part compared the value of ground soy beans with linseed oilmeal for milk and fat production.

TABLE 1

*Chemical analyses of protein supplements used*

| TRIAL | CONCENTRATE | CHEMICAL ANALYSES | | | | | |
|---|---|---|---|---|---|---|---|
| | | Moisture | Ash | Crude protein | Crude fiber | Nitrogen free extract | Fat |
| | Linseed oilmeal (Old process) | 9.1 | 5.4 | 33.9 | 8.4 | 35.7 | 7.5 |
| I | Soy bean oilmeal (Old process) | 7.2 | 5.4 | 44.5 | 6.9 | 29.3 | 6.7 |
| II | Soy bean oilmeal (New process) | 6.6 | 7.1 | 44.5 | 7.2 | 32.4 | 2.2 |
| III | Soy bean oilmeal (Old process) | 9.1 | 5.7 | 35.8 | 6.6 | 37.0 | 5.7 |
| IV | Ground soy beans | 7.5 | 5.4 | 32.2 | 11.0 | 32.5 | 11.4 |
| V | Ground soy beans | Same as trial IV | | | | | |

The chemical analyses of the various protein supplements used in the experiment show the nutrient content given in table 1.

## CONDUCT OF THE EXPERIMENT

Each trial of this experiment consisted of three twenty-eight-day periods which included a seven-day preliminary period, thus making an actual experimental feeding period of twenty-one days. The reversal method of feeding was used throughout these trials.

### *Production records*

The milk produced by each cow was weighed and recorded after each milking, and composite samples, containing proportionate amounts of milk from each milking of each cow, were made and tested for butterfat content, every seven days.

### *Feeds*

*Grain.* The grain rations fed throughout this experiment were the ordinary herd mixtures and were not uniform throughout the entire five trials. The grain ration during each trial, however, remained the same, with the exception of the changes in the high protein supplements, so that a direct comparison could be made between these supplements.

TABLE 2

*Results of trial I*

| PERIOD | CONCENTRATE | MILK PRODUCTION | FAT | PER CENT FAT |
|---|---|---|---|---|
| | | *pounds* | *pounds* | |
| 1 | Soy bean oilmeal | 2178.5 | 76.5 | 3.54 |
| 3 | Soy bean oilmeal | 2150.2 | 78.5 | 3.65 |
| Average | | 2164.3 | 77.5 | 3.59 |
| 2 | Linseed oilmeal | 2169.1 | 75.5 | 3.5 |

*Roughage.* Silage was carefully weighed to the cows at the rate of 3 pounds per day per 100 pounds body weight. Alfalfa hay was fed to the cows at the rate of 1 pound per day per 100 pounds body weight.

The grain ration was fed at the rate of 1 pound for every 3 pounds of 4 to 6 per cent milk produced and 1 pound for every 4 pounds of 3 to 4 per cent milk produced.

### *Trial I*

In this trial, a comparison was made between old process linseed oilmeal and old process soybean oilmeal. The feeds were fed to three lots of cows, with three cows in each lot, one Jersey and two Ayrshires or two Holsteins in each lot, so that the milk production and fat test would be similar. Table 2 shows the average amounts of milk and fat produced during each of the three feeding periods. The average production of milk and fat of the first and third periods, during which time the cows were fed soy bean oilmeal, is compared with the production of the second period, when the cows were fed linseed oilmeal.

From table 2 it can be seen that the average milk production resulting from each protein supplement fed was almost identical. The fat test, however, seemed to favor the soy bean meal over the linseed oilmeal by approximately 0.1 per cent. This gave a higher total fat production by approximately 2.5 per cent, when soy bean oilmeal was fed than when linseed oilmeal was fed.

TABLE 3

*Results of trial II*

| PERIOD | CONCENTRATE | MILK PRODUCTION | FAT | PER CENT FAT |
|---|---|---|---|---|
| | | *pounds* | *pounds* | |
| 1 | Soy bean oilmeal | 2490.6 | 77.71 | 3.120 |
| 3 | Soy bean oilmeal | 2222.6 | 66.29 | 2.982 |
| Average | | 2356.6 | 72.00 | 3.051 |
| 2 | Linseed oilmeal | 2335.0 | 74.46 | 3.188 |

## *Trial II*

In this trial, comparison was made between new process soy bean oilmeal and old process linseed oilmeal in regard to milk and fat production. Two lots of cows, with four cows in each lot, were fed by the reversal method. The data obtained is shown in table 3.

These figures indicate that the soy bean oilmeal caused approximately 1 per cent greater milk production while the linseed oilmeal produced approximately 3 per cent more fat than the soy bean oilmeal.

### *Trial III*

In trial III, old process soy bean oilmeal was compared with old process linseed oilmeal. The cows in these lots, three cows in each lot, were allowed to run on pasture and during the latter part of the trial were given free access to racks of alfalfa hay. Table 4 shows the amount of milk and fat produced and the average fat test according to the protein concentrate fed.

These figures show that soy bean oilmeal produced slightly more milk (1.25 per cent) than the linseed oil meal while the fat production was practically the same, regardless of the kind of protein supplement fed. The average fat test of the lots receiving soy bean oilmeal was practically the same as that of the lot receiving linseed oilmeal.

TABLE 4

*Results of trial III*

| PERIOD | CONCENTRATE | MILK PRODUCTION | FAT | PER CENT FAT |
|---|---|---|---|---|
| | | *pounds* | *pounds* | |
| 1 | Soy bean oilmeal | 2214.3 | 70.8 | 3.19 |
| 3 | Soy bean oilmeal | 2270.2 | 69.01 | 3.04 |
| Average | | 2242.2 | 69.9 | 3.115 |
| 2 | Linseed oilmeal | 2213.4 | 69.35 | 3.13 |

*Summary.* A general summary of these three feeding trials comparing soy bean oilmeal with linseed oilmeal showed:

1. That old process soy bean oilmeal gave better results than new process soy bean oilmeal.

2. Both kinds of soy bean oilmeal gave better results than linseed oilmeal in milk production.

3. New process soy bean oilmeal gave decidedly lower production in fat than old process soy bean oil meal and somewhat lower production in fat than linseed oilmeal.

4. That a correlation apparently exists between the chemical analyses of the concentrates and the amount of fat produced, as new process soy bean oilmeal (which was a chemically extracted meal) showed a decidedly lower percentage of fat than either old

process soy bean oilmeal or old process linseed oilmeal. The average fat test of the cows receiving new process soy bean oil meal was decidedly lower—almost 0.2 per cent—than that of the cows receiving linseed oilmeal.

5. The average fat test of the cows receiving old process soy bean oilmeal was slightly higher than that of those receiving linseed oilmeal.

### *Trial IV*

In this trial, a comparison was made between two lots of four cows in each lot, to determine the relative feeding value of ground soy beans and old process linseed oilmeal. The beans used in this trial were not whole beans but were small and broken beans cleaned from soy beans that were to be used for seed. The average percentage composition of the soy beans showed that they were decidedly higher in fat but lower in protein than either the soy bean oilmeal or linseed oilmeal.

TABLE 5

*Results of trial IV*

| PERIOD | CONCENTRATE | MILK PRODUCTION | FAT | PER CENT FAT |
|---|---|---|---|---|
| | | *pounds* | *pounds* | |
| 1 | Ground soy beans | 2440.7 | 87.71 | 3.59 |
| 3 | Ground soy beans | 2317.7 | 82.68 | 3.56 |
| Average | | 2379.2 | 85.19 | 3.58 |
| 2 | Linseed oilmeal | 2318.8 | 83.18 | 3.58 |

The average production of these two lots when fed ground soy beans as compared with linseed oilmeal is as shown in table 5.

These figures show that ground soy beans produced approximately 2.5 per cent more milk and from 2 to 2.5 per cent more fat than the linseed oilmeal. The fat test was the same, regardless of the protein concentrate fed.

### *Trial V*

This trial was conducted with two lots of three lots each. The method of comparison was the same as in the other trials. The figures in table 6 show the average production of milk and fat and the average percentage of fat of the two lots of cows when fed ground soy beans as compared with linseed oilmeal.

These figures are also in favor of the ground soy beans, there being a difference of approximately 3 per cent, in milk production, and 2.5 to 3 per cent in fat production in favor of the ground soy beans.

*Summary.* The data obtained in trials IV and V, when ground soy beans were compared with linseed oilmeal show:

1. That there was an average increase of between 2 to 3 per cent in both milk and fat production in favor of ground soy beans when compared with linseed oilmeal.

TABLE 6

*Results of trial V*

| PERIOD | CONCENTRATE | MILK PRODUCTION | FAT | PER CENT FAT |
|---|---|---|---|---|
| | | *pounds* | *pounds* | |
| 1 | Ground soy beans | 1825.1 | 55.48 | 3.01 |
| 3 | Ground soy beans | 1735.6 | 52.2 | 3.00 |
| Average | | 1780.3 | 53.84 | 3.024 |
| 2 | Linseed oilmeal | 1723.9 | 52.30 | 3.053 |

2. While the fat content of the ground soy beans was decidedly higher than that of the linseed oilmeal, the average butterfat test of the milk produced by cows receiving each of these protein supplements was practically identical.

## CONCLUSIONS

The results of this experiment to determine the feeding value of soy bean oilmeal and ground soy beans compared with linseed oil meal as a protein supplement in the dairy ration show:

1. That soy bean oilmeal has a feeding value equal to that of linseed oilmeal.

2. That ground soy beans have a feeding value for milk and fat production from 2 to 3 per cent higher than linseed oilmeal.

3. That ground soy beans, while much lower in protein than soy bean oilmeal, contain a much greater percentage of fat and from this source probably obtain much of their feeding value.
4. Ground soy beans can become one of the best sources for protein in the ration of Indiana dairy cows.

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# A BACTERIOLOGICAL STUDY OF THE HOMOGENIZING PROCESS IN MAKING ICE CREAM[1]

FREDERICK W. FABIAN

*Department of Bacteriology and Hygiene, Michigan Agricultural Experiment Station, East Lansing, Michigan*

## INTRODUCTION

In a previous paper (1) a bacteriological study was made of the influence of various manufacturing operations upon the bacterial content of ice cream. One of the operations studied was homogenizing. In this operation, which takes but a short time to complete, an increase in bacterial count was noted in the majority of the samples studied. The increase noted in the mix was presumed to be due to two causes: First, bacterial contamination of the mix from the homogenizer, and, second, a breaking up of the clumps of bacteria as the mix passed through the homogenizer. It was to determine whether there is an actual breaking up of the bacterial clumps as the ice cream mix passes through the homogenizer that the present work was undertaken.

## PREVIOUS WORK

Hammer and Sanders (2) made a bacteriological study of the influence of homogenizing the ice cream mix upon the bacterial count both with and without pressure. When the mix was run through the homogenizer without pressure, they found an increase in the numbers of bacteria in all cases. However, all the samples analyzed were from the first material passing through. This material would serve to partially free the machine from bacterial contamination so that subsequent material passing through should not be contaminated from this source to nearly as great an extent. When the mix was passed through the homogenizer under pressure, an increase in the number of bacteria was shown in all cases except one. The increase in the number of bacteria when the mix was homogenized under pressure was not as great in most cases as without pressure. They state that, "At least two factors are operating to change the bacterial content when the pressure is thrown on; first, the machine has been in operation longer and the contamination from it is becoming less; second, the agitation in the machine tends to break up any clumps that may be present and thus apparently increase the count."

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[1] Published by permission of the Director of the Michigan Experiment Station, East Lansing, Mich. Received for publication December 1, 1924.

Submitted in partial fulfilment of the requirement for M.S. degree. Michigan Agricultural College, East Lansing, Mich.

Peterson and Tracy (3) in a study to determine the relative importance of each step in the manufacture of the mix say that, "The increase after homogenizing and freezing is probably due, for the most part, to the breaking up of the bacterial clusters, which results in a higher count by the plate method."

Fay and Olson (10) in their study of the homogenizing process state that, "In general, however, the increases were larger than the decreases, and the averages of all the counts showed an increase of 26 per cent after homogenizing. Such an increase in bacterial count is, no doubt, more apparent than real, being due chiefly to the breaking up of clusters of organisms each individual of which may give rise to a separate colony on an agar plate." From their study of this process they conclude that, "Homogenization of the mix usually causes an increase in the bacterial count as determined by the agar-plate method. Such an increase is probably due to the breaking up of clumps of bacteria."

## PRESENT WORK

The present work was designed to determine whether there was a breaking up of the bacterial clumps during the process of homogenizing. The materials going into the ice cream mix were placed in a starter can and heated to 145°F. for thirty minutes. The mix was thoroughly stirred by mechanical paddles during the entire pasteurizing process which usually took from forty-five minutes to an hour to complete. After the mix had been pasteurized it was then passed through the homogenizer at 2000 pounds pressure. Samples were taken as the mix passed into the homogenizer and as it came out on the other side. The samples were always collected after about half the mix had passed through the homogenizer or toward the end but never at the beginning since it was desirable to reduce the bacterial contamination from the homogenizer to a minimum.

### *Method*

The samples were analyzed by two methods; the plate method and the direct microscopic method. The numbers of bacteria by the plate method were determined as follows: 1 gram of the mix was weighed into an Erlenmeyer flask and 99 cc. of sterile physiological saline solution added to it. Suitable dilutions were made from this and plated on milk-powder agar (4). The dilutions used were 1:100; 1:1000 and 1:10,000. The plates were incubated at 37°C. and counted at the end of forty-eight hours. All plates were made in duplicate and the counts represent an average of the two best plates unless otherwise noted. All counts are therefore per gram and not per cubic centimeter.

Breed's direct microscopic method (5), (6), (7), (8) with slight modifications was used to determine the numbers of individual and of groups of bacteria present. The method used was as follows: 0.01 gram of the mix was weighed on each end of a clean slide, and spread evenly over an area of 1 square cm. The slides were made in duplicate so that for each sample of mix four 1 square cm. areas were made. After the slides had been dried they were immersed in xylol for five minutes to remove the fat. The surplus xylol was removed and the slides dried again. They were then fixed in 95 per cent alcohol for twenty minutes and dried. After this they were stained for thirty seconds in Loeffler's alkaline methylene blue and decolorized to a light blue in 95 per cent alcohol. The bacteria stained a deeper blue than the rest of the material. Because of the larger amount of fat present in ice cream the fields are more pitted than in the case of milk. However, satisfactory preparations for comparison and other detailed information may be obtained. Twenty-five or more fields from each area were counted and averaged. This made a total of 100 fields for each sample. The counting was done under a 1.9 mm. oil-immersion lens. The factor used was 500,000 and was derived as follows: Diameter of the field was 0.16 mm. By substituting in the following equation

$$\frac{x}{\pi R^2} \times 100 = y$$

where $x$ equals the area of the smear in square millimeters, $R$ the radius of the field in millimeters whence $\pi R^2$ equals the area, and $y$ equals the factor necessary to transform the number of bacteria found in one field of the microscope into terms of bacteria per gram. Substituting we get

$$\frac{1 \times 100}{3.1416 \times (0.08)^2} \times \frac{100}{1} = 500{,}000$$

Since a hundred or more fields from each sample were counted, it was necessary to find the average per field and multiply this average by the factor. This was done as follows:

$$\frac{500{,}000}{n} \times m = \text{number of bacteria per gram}$$

when $n$ = the number of fields counted and $m$ the total number of bacteria found in $n$ fields.

An explanation is necessary of the terms "individuals" and "groups." By the term "individuals" is meant the total number of bacteria including isolated single bacteria and the individual bacteria in groups (any organism in the process of division was counted as two individuals); thus the count under the column labeled individuals represents a total of all the bacterial cells in an average of 100 fields times the factor. The column labeled "groups" represents a somewhat different meaning than is usually ascribed to this term and should be thoroughly understood by anyone wishing to interpret the data correctly. By this term is meant those bacteria, either individuals or groups, so located in the microscopic field that in the opinion of the observer would, if alive and plated on suitable medium, form one bacterial colony.

Thus a single individual if sufficiently isolated would count as a "group" or several individuals if sufficiently close to each other would constitute a "group." In the process of counting many objects were encountered about which there might be a reasonable doubt as to whether they were bacteria or not. In all such cases, they were not counted as bacteria.

TABLE 1

*Comparison of plate count and direct microscopic count before and after homogenizing*

| DATE | PLATE COUNT | | DIRECT MICROSCOPIC COUNT | | | |
|---|---|---|---|---|---|---|
| | Before | After | Before | | After | |
| | | | Individuals | "Groups" | Individuals | "Groups" |
| *1922* | | | | | | |
| 10/3 | 5,800 | 10,000 | 1,980,000 | 1,160,000 | 3,330,000 | 3,110,000 |
| 10/10 | 17,000 | 18,000 | 1,950,000 | 945,000 | 2,200,000 | 2,000,000 |
| 10/17 | 10,000 | 25,000 | 1,450,000 | 1,100,000 | 7,335,000 | 7,120,000 |
| 10/23 | 46,500 | 71,000 | 2,400,000 | 1,890,000 | 3,000,000 | 2,900,000 |
| 10/30 | 12,500 | 31,000 | 2,865,000 | 2,200,000 | 3,825,000 | 3,450,000 |
| 11/3 | 7,600 | 16,000 | 3,400,000 | 2,500,000 | 4,100,000 | 3,800,000 |
| 11/10 | 18,000 | 30,000 | 2,780,000 | 1,650,000 | 4,350,000 | 4,000,000 |
| 11/15 | 6,500 | 23,000 | 2,800,000 | 1,650,000 | 2,700,000 | 2,320,000 |
| 12/20 | 19,000 | 88,000 | 2,500,000 | 1,560,000 | 4,315,000 | 4,000,000 |
| *1923* | | | | | | |
| 3/16 | 8,100 | 4,600 | 2,400,000 | 1,550,000 | 3,725,000 | 3,230,000 |
| 3/21 | 23,000 | 67,000 | 3,340,000 | 1,880,000 | 3,885,000 | 3,600,000 |
| 4/10 | 4,500 | 8,000 | 2,330,000 | 1,700,000 | 3,000,000 | 2,565,000 |
| 4/18 | 5,500 | 15,000 | 2,530,000 | 1,585,000 | 2,680,000 | 2,195,000 |
| 4/19 | 12,000 | 18,000 | 3,000,000 | 2,600,000 | 2,720,000 | 2,340,000 |
| 10/8 | 16,000 | 38,000 | 1,700,000 | 1,560,000 | 1,850,000 | 1,700,000 |
| 10/12 | 3,000 | 3,800 | 1,600,000 | 1,300,000 | 1,900,000 | 1,700,000 |
| 10/19 | 24,000 | 83,000 | 490,000 | 455,000 | 465,000 | 435,000 |
| 10/25 | 14,000 | 60,000 | 850,000 | 750,000 | 1,600,000 | 1,500,000 |
| 11/9 | 69,000 | 75,000 | 545,000 | 450,000 | 720,000 | 600,000 |
| 11/13 | 6,500 | 19,000 | 2,000,000 | 1,500,000 | 2,200,000 | 2,000,000 |
| 11/15 | 3,600 | 6,500 | 665,000 | 570,000 | 570,000 | 555,000 |
| 11/22 | 12,500 | 170,000 | 3,965,000 | 2,000,000 | 2,420,000 | 2,160,000 |
| 11/28 | 11,000 | 45,000 | 2,100,000 | 1,600,000 | 3,500,000 | 3,200,000 |
| 12/3 | 2,500 | 300,000 | 1,850,000 | 1,450,000 | 3,500,000 | 3,200,000 |
| *1924* | | | | | | |
| 2/20 | 4,400 | 6,000 | 2,665,000 | 1,440,000 | 2,700,000 | 1,750,000 |

## *Results*

The results of the experiment are set forth in the tables that follow. In table 1 a comparison is made of the data secured from the plate count and the direct microscopic count. In the second column under plate count are listed the number of bacteria found in the ice cream mix before it was homogenized, but after it had been pasteurized at 145°F. for thirty minutes. In the third column are listed the numbers of bacteria found on the plates after the ice cream mix had passed through the homogenizer under 2000 pounds pressure.

In column 4 are listed the counts of individual bacteria of the same mix under the same conditions as in column 2 above except that the number of bacteria have been determined by the modified direct microscopic method. In column 5 are listed the "groups" of bacteria of this same mix as determined by the modified microscopic method.

In columns 6 and 7 are listed respectively the counts of the same mix as listed in column 3 above except that the "individuals" and "groups" have been determined by the direct microscopic method slightly modified. In other words the same mix under identical conditions has been analyzed by two different bacteriological methods both before and after the mechanical operation of homogenizing to determine the effect this process had on the bacterial content of the mix. A comparison of the plate count and direct microscopic count before and after homogenizing are set forth in table 1.

## *Discussion*

A comparison of columns 2 and 3 of table 1 shows that there is an increase in the number of bacteria after homogenizing as compared with before homogenizing as determined by the plate count in all cases except one. In some cases the increase is large while in other cases it is small.

A comparison of columns 4 and 6 tabulating individuals before and after homogenizing, as determined by the direct microscopic method, shows, that in 20 out of 25 or 80 per cent of the samples there is an increase; while in 5 out of 25 or 20 per cent of the samples, there is a decrease in the number of individuals after as compared with before homogenizing. The same relationship as just stated for individuals also holds true for "groups" tabulated in columns 5 and 7. It will thus be seen that, in the majority of cases, there is an increase in the bacterial count, as determined by both the plate and direct microscopic method, after the mix has passed through the homogenizer.

### RELATIONSHIP BETWEEN INDIVIDUALS, "GROUPS" AND GROUP SIZE

The individuals, "groups," and group size are recorded in table 2. What is meant by individuals and "groups" has been defined earlier in this paper. However, it was thought advisable to make further tabulations concerning the number of groups and group size, since we are concerned primarily in trying to determine the cause of the increase of the number of bacteria as determined by the plate count and as reported by previous investigators.

For this reason a record was made of the number of groups and the size of each group as the count was made. In column 7 are tabulated all the groups of two and in column 8 all the groups of three or more. Total groups of two or more are listed in column 6 while the average size of groups of two or more are listed in column 9. The complete record of this detailed information is given in table 2.

### *Discussion*

The size of groups most common above two are three, four and five in the order mentioned. Groups of eight are not infrequent while the size of a few groups ran as large as sixty or more. However, the larger groups occurred rather infrequently in the ice cream mix.

It will be noticed that there is a considerable difference between the average size of "groups" in column 5 and the average size of groups of two or more in column 9, the average size of groups in column 9 being considerably larger than in column 2. This is

TABLE 2

*Tabulation of individuals, "groups" and group size*

| DATE | | NUMBER OF INDIVIDUAL BACTERIA | NUMBER OF "GROUPS" | AVERAGE SIZE OF "GROUPS" | TOTAL GROUPS OF TWO OR MORE | NUMBER OF | | AVERAGE SIZE OF GROUPS OF TWO OR MORE |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Groups of two | Groups of three or more | |
| *1922* | | | | | | | | |
| 10/ 3 | B* | 1,980,000 | 1,160,000 | 1.70 | 39 | 19 | 20 | 3.8 |
| | A | 3,330,000 | 3,110,000 | 1.07 | 28 | 17 | 11 | 3.0 |
| 10/10 | B | 1,950,000 | 945,000 | 2.06 | 55 | 26 | 29 | 4.7 |
| | A | 2,200,000 | 2,000,000 | 1.10 | 39 | 34 | 5 | 2.3 |
| 10/17 | B | 1,450,000 | 1,100,000 | 1.31 | 30 | 11 | 19 | 3.0 |
| | A | 7,335,000 | 7,120,000 | 1.03 | 14 | 4 | 10 | 2.9 |
| 10/23 | B | 2,400,000 | 1,890,000 | 1.27 | 44 | 16 | 28 | 5.0 |
| | A | 3,000,000 | 2,900,000 | 1.04 | 5 | 3 | 2 | 2.4 |
| 10/30 | B | 2,865,000 | 2,200,000 | 1.32 | 78 | 31 | 47 | 3.0 |
| | A | 3,825,000 | 3,450,000 | 1.17 | 40 | 27 | 13 | 2.5 |
| 11/ 3 | B | 3,400,000 | 2,500,000 | 1.36 | 65 | 19 | 46 | 3.8 |
| | A | 4,100,000 | 3,800,000 | 1.06 | 41 | 21 | 20 | 2.4 |
| 11/10 | B | 2,780,000 | 1,650,000 | 1.70 | 106 | 34 | 72 | 3.5 |
| | A | 4,350,000 | 4,000,000 | 1.08 | 35 | 16 | 19 | 2.8 |
| 11/15 | B | 2,800,000 | 1,650,000 | 1.70 | 104 | 33 | 71 | 4.0 |
| | A | 2,700,000 | 2,320,000 | 1.16 | 23 | 10 | 13 | 3.0 |
| 12/20 | B | 2,520,000 | 1,560,000 | 1.42 | 92 | 45 | 47 | 3.1 |
| | A | 4,315,000 | 4,000,000 | 1.07 | 38 | 20 | 18 | 2.6 |
| *1923* | | | | | | | | |
| 3/16 | B | 2,400,000 | 1,550,000 | 1.54 | 95 | 32 | 62 | 3.1 |
| | A | 3,725,000 | 3,230,000 | 1.15 | 58 | 30 | 28 | 2.3 |
| 3/21 | B | 3,340,000 | 1,880,000 | 1.77 | 78 | 40 | 38 | 3.3 |
| | A | 3,885,000 | 3,600,000 | 1.08 | 42 | 29 | 13 | 2.4 |
| 4/10 | B | 2,330,000 | 1,700,000 | 1.43 | 76 | 44 | 32 | 3.0 |
| | A | 3,000,000 | 2,565,000 | 1.17 | 60 | 39 | 21 | 2.4 |
| 4/18 | B | 2,530,000 | 1,585,000 | 1.69 | 89 | 40 | 49 | 3.2 |
| | A | 2,680,000 | 2,195,000 | 1.22 | 36 | 27 | 9 | 3.1 |
| 4/19 | B | 3,000,000 | 2,600,000 | 1.15 | 46 | 27 | 19 | 3.0 |
| | A | 2,720,000 | 2,340,000 | 1.11 | 51 | 37 | 14 | 2.5 |

TABLE 2—*Continued*

| DATE | | NUMBER OF INDIVIDUAL BACTERIA | NUMBER OF "GROUPS" | AVERAGE SIZE OF "GROUPS" | TOTAL GROUPS OF TWO OR MORE | NUMBER OF | | AVERAGE SIZE OF GROUPS OF TWO OR MORE |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Groups of two | Groups of three or more | |
| *1923* | | | | | | | | |
| 10/ 8 | B | 1,700,000 | 1,560,000 | 1.09 | 30 | 27 | 3 | 2.2 |
| | A | 1,850,000 | 1,700,000 | 1.08 | 23 | 20 | 3 | 2.1 |
| 10/12 | B | 1,600,000 | 1,300,000 | 1.23 | 44 | 35 | 9 | 2.2 |
| | A | 1,900,000 | 1,700,000 | 1.11 | 29 | 24 | 5 | 2.1 |
| 10/19 | B | 490,000 | 455,000 | 1.07 | 17 | 16 | 1 | 2.2 |
| | A | 465,000 | 435,000 | 1.06 | 9 | 8 | 1 | 2.1 |
| 10/25 | B | 850,000 | 750,000 | 1.13 | 27 | 21 | 6 | 2.3 |
| | A | 1,600,000 | 1,500,000 | 1.06 | 21 | 21 | 0 | 2.0 |
| 11/ 9 | B | 545,000 | 450,000 | 1.21 | 18 | 17 | 1 | 2.1 |
| | A | 720,000 | 600,000 | 1.20 | 25 | 23 | 2 | 2.0 |
| 11/13 | B | 2,000,000 | 1,500,000 | 1.33 | 67 | 61 | 6 | 2.4 |
| | A | 2,200,000 | 2,000,000 | 1.10 | 37 | 36 | 1 | 2.0 |
| 11/15 | B | 665,000 | 570,000 | 1.17 | 15 | 13 | 2 | 2.5 |
| | A | 570,000 | 555,000 | 1.02 | 20 | 15 | 5 | 2.2 |
| 11/22 | B | 3,965,000 | 2,000,000 | 1.98 | 173 | 127 | 46 | 4.3 |
| | A | 2,420,000 | 2,160,000 | 1.12 | 33 | 33 | 0 | 2.0 |
| 11/28 | B | 2,100,000 | 1,600,000 | 1.31 | 99 | 87 | 12 | 2.1 |
| | A | 3,200,000 | 2,950,000 | 1.06 | 63 | 61 | 2 | 2.0 |
| 12/ 3 | B | 1,850,000 | 1,450,000 | 1.27 | 72 | 66 | 6 | 2.1 |
| | A | 3,500,000 | 3,200,000 | 1.09 | 44 | 43 | 1 | 2.0 |
| *1924* | | | | | | | | |
| 2/20 | B | 2,665,000 | 1,440,000 | 1.85 | 112 | 76 | 36 | 3.1 |
| | A | 2,700,000 | 1,750,000 | 1.54 | 105 | 76 | 29 | 2.4 |

* B = before mix passed through homogenizer; A = after mix passed through homogenizer.

readily understood when the method of computing these averages is explained.

The method of computing the average size of the "group" in column 5 was by dividing the total number of individuals by the total number of "groups;" while the method used in computing the average size of the groups of two or more in column 9 was by finding the total number of individuals occurring in the groups alone (not including separate individuals) and dividing this sum by the total number of actual groups. Both methods of computation show that there is a decrease in the size of groups after the mix has passed through the homogenizer.

Since all these differences are of the same sign, it is safe to conclude that homogenizing reduces the average size of the clumps of bacteria in the mix.

## A STUDY OF THE INFLUENCE OF HOMOGENIZING ON THE SIZE OF THE BACTERIAL CLUMPS IN MILK

When the work had been completed showing the influence of homogenizing on ice cream, it was thought that a similar study of a few samples of milk might prove interesting and throw some light on the problem. Accordingly 5 samples of milk were studied with this in view. At first it was thought advisable to secure samples of fresh unpasteurized milk having large numbers of bacteria present.

Samples of milk coming into the dairy were analyzed until one was found with a high bacterial plate count. This was run but it was not found to be satisfactory for this work as will be seen later. A composite sample of milk as delivered at the dairy was found most satisfactory and was used in the other 4 cases.

### *Procedure*

A 10-gallon can of fresh unpasteurized milk containing milk from many different farms was taken from the vat at the College dairy. This can of milk was then dumped into the starter can leading to the homogenizer and a sample taken. It was then passed through the homogenizer without pressure. The homogenizer pressure was then raised to 1000, 1500, 2000 and 2500 pounds pressure per square inch respectively and samples taken as it was leaving the homogenizer at these various pressures.

These samples were then taken immediately to the laboratory and analyzed according to the direct and plate methods as previously described. Previous to homogenization, the homogenizer was rinsed with sterile water and a sample of this rinse water was collected and analyzed by the plate method.

TABLE 3

*Sample 1, February 28, 1924*

| OPERATION | PLATE COUNT | NUMBER OF INDIVIDUAL BACTERIA | NUMBER OF "GROUPS" | AVERAGE SIZE OF "GROUPS" | REMARKS |
|---|---|---|---|---|---|
| A | 3,500,000 | 50,000,000* | 2,520,000 | 19.84 | 25 fields |
| B | 19,000,000 | 166,300,000* | Bacteria spread evenly over entire field | | |
| C | 14,000,000 | | Bacteria spread evenly over entire field | | |
| D | 26,000,000 | | Bacteria spread evenly over entire field | | |
| E | 23,000,000 | | Bacteria spread evenly over entire field | | |
| F | 35,400,000† | | Bacteria spread evenly over entire field | | |

Water from homogenizer, 1500 bacteria per cubic centimeter.

* Twenty-five fields counted.

† Only one plate counted.

*Explanation of letters for all tables*

Operation A = fresh unpasteurized milk from vat.
Operation B = same milk passed through homogenizer without pressure.
Operation C = same milk passed through homogenizer at 1000 pounds pressure.
Operation D = same milk passed through homogenizer at 1500 pounds pressure.
Operation E = same milk passed through homogenizer at 2000 pounds pressure.
Operation F = same milk passed through homogenizer at 2500 pounds pressure.

TABLE 4

*Sample 2, March 13, 1924*

| OPERATION | PLATE COUNT | NUMBER OF INDIVIDUAL BACTERIA | NUMBER OF "GROUPS" | AVERAGE SIZE OF "GROUPS" | TOTAL GROUPS OF TWO OR MORE | NUMBER OF | | AVERAGE SIZE OF GROUPS |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Groups of two | Groups of three or more | |
| A | 840,000 | 2,700,000 | 1,240,000 | 2.17 | 142 | 85 | 57 | 2.8 |
| B | 1,000,000 | 4,500,000 | 1,580,000 | 2.84 | 154 | 64 | 90 | 5.0 |
| C | 2,115,000 | 2,570,000 | 1,800,000 | 1.43 | 101 | 72 | 29 | 2.5 |
| D | 2,300,000 | 2,400,000 | 1,330,000 | 1.80 | 97 | 69 | 28 | 2.5 |
| E | 2,600,000 | 1,830,000 | 1,380,000 | 1.32 | 78 | 64 | 14 | 2.2 |
| F | 2,800,000 | 2,500,000 | 1,450,000 | 1.73 | 77 | 54 | 23 | 2.4 |

Water from homogenizer, 6 bacteria per cubic centimeter.

TABLE 5

*Sample 3, March 20, 1924*

| OPERATION | PLATE COUNT | NUMBER OF INDIVIDUAL BACTERIA | NUMBER OF "GROUPS" | AVERAGE SIZE OF "GROUPS" | TOTAL GROUPS OF TWO OR MORE | NUMBER OF | | AVERAGE SIZE OF GROUPS |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Groups of two | Groups of three or more | |
| A | 25,000 | 450,000* | 320,000 | 1.40 | 5 | 4 | 1 | 3.6 |
| B | 68,000 | 550,000 | 300,000 | 1.83 | 19 | 15 | 4 | 2.5 |
| C | 124,000 | 360,000 | 290,000 | 1.24 | 10 | 9 | 1 | 2.3 |
| D | 78,000 | 340,000 | 295,000 | 1.15 | 8 | 7 | 1 | 2.1 |
| E | 77,000 | 350,000 | 315,000 | 1.11 | 7 | 7 | 0 | 2.0 |
| F | 86,000 | 350,000* | 300,000 | 1.16 | 3 | 3 | 0 | 2.0 |

Water from homogenizer, 125,000 bacteria per cubic centimeter.

* Fifty fields counted.

TABLE 6

*Sample 4, March 26, 1924*

| OPERATION | PLATE COUNT | NUMBER OF INDIVIDUAL BACTERIA | NUMBER OF "GROUPS" | AVERAGE SIZE OF "GROUPS" | TOTAL GROUPS OF TWO OR MORE | NUMBER OF | | AVERAGE SIZE OF GROUPS |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Groups of two | Groups of three or more | |
| A | 435,000 | 1,870,000 | 675,000 | 2.77 | 38 | 33 | 15 | 6.8 |
| B | 2,300,000 | 3,500,000 | 1,500,000 | 2.33 | 182 | 119 | 63 | 3.1 |
| C | 2,200,000 | 4,200,000 | 2,400,000 | 1.75 | 194 | 123 | 71 | 2.8 |
| D | 2,600,000 | 4,500,000 | 2,400,000 | 1.87 | 220 | 135 | 85 | 2.9 |
| E | 2,800,000 | 3,000,000 | 2,320,000 | 1.29 | 145 | 102 | 43 | 2.5 |
| F | 3,400,000 | 2,300,000 | 1,460,000 | 1.57 | 120 | 67 | 43 | 2.5 |

Water from homogenizer, 140,000 bacteria per cubic centimeter.

TABLE 7

*Sample 5, March 27, 1924*

| OPERATION | PLATE COUNT | NUMBER OF INDIVIDUAL BACTERIA | NUMBER OF "GROUPS" | AVERAGE SIZE OF "GROUPS" | TOTAL GROUPS OF TWO OR MORE | NUMBER OF | | AVERAGE SIZE of GROUPS |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Groups of two | Groups of three or more | |
| A | 250,000 | 1,950,000 | 780,000 | 2.5 | 39 | 21 | 18 | 7.0 |
| B | 400,000 | 2,400,000 | 800,000 | 3.0 | 89 | 53 | 36 | 4.9 |
| C | 72,000 | 1,700,000 | 875,000 | 1.94 | 71 | 40 | 31 | 3.2 |
| D | 105,000 | 1,400,000 | 830,000 | 1.70 | 69 | 54 | 15 | 2.5 |
| E | 285,000 | 1,400,000 | 950,000 | 1.47 | 56 | 37 | 19 | 2.6 |
| F | 560,000 | 1,700,000 | 1,000,000 | 1.70 | 56 | 34 | 22 | 2.1 |

Water from homogenizer, 1800 bacteria per cubic centimeter.

### *Results*

The results of this experiment are recorded in tables 3 to 7. In table 3 are recorded the results obtained by using the sample of milk containing a large number of bacteria. Only 25 fields were counted because the bacteria were so numerous. In this sample there were so many large clumps and the organisms so many that the number of bacteria in the large clumps had to be estimated. For this reason it was thought best to work with milk containing fewer bacteria. This would enable a more accurate counting of the bacteria in the groups.

In tables 4, 5, 6 and 7 are recorded the results from the composite samples of milk. The numbers of bacteria by the plate count and the direct microscopic count are recorded the same as for the ice cream mix. The same information as to individuals, "groups," average size of "groups," etc., is also contained in the tables.

The operations are lettered and have the following meaning: Operation "A," fresh unpasteurized milk after being placed in the starter can and just before passing through the homogenizer; operation "B," the same milk after passing through the homogenizer without pressure; operation "C," same milk after passing through the homogenizer at 1000 pounds pressure; operation "D," same milk after passing through the homogenizer at 1500 pounds pressure; operation "E," same milk after passing through the homogenizer at 2000 pounds pressure; operation "F," same milk after passing through the homogenizer at 2500 pounds pressure.

A detailed record of the results is set forth in tables 3 to 7.

## INFLUENCE OF HOMOGENIZING ON THE SIZE OF THE BACTERIAL CLUMPS IN MILK

### *Discussion*

A careful study of the data shows that the clumps of bacteria in milk are broken up during the process of homogenizing. This is especially marked in the first sample of milk run. In this sample large clumps of bacteria together with many smaller clumps were scattered throughout the entire smear before the milk was homogenized. After homogenizing, however, the bacteria were evenly distributed throughout the entire smear and the size of the clumps greatly reduced in size. The same held true for the other samples homogenized but to a less degree because there were fewer large groups and not nearly as many organisms present. In all cases where the milk passed through the homogenizer under pressure there is a decrease in the size of the groups.

The data also show that the homogenizer is a source of contamination for in all cases there is an increase in both the plate and microscopic counts after the milk has passed through the homogenizer. It is true that a part of this increase might be attributed to the breaking up of clumps and no doubt was to a slight extent but the large increase should then be maintained throughout which is not the case. Another interesting thing is that the average size of the "groups" increases in most cases after the milk has passed through the homogenizer, which would indicate contamination from the homogenizer. The average size of groups decreases slightly in most cases with an increase of pressure. The decrease is gradual which would indicate that as the groups get smaller they are harder to break up.

The above data show that homogenizing has the same influence on the bacterial groups in milk as it does on the bacteria groups in the ice cream mix.

## A STUDY OF THE TYPES OF BACTERIA FOUND IN THE HOMOGENIZER AND IN THE ICE CREAM MIX BEFORE AND AFTER HOMOGENIZING

In making a bacteriological study of the homogenizing process milk-powder agar was used. By the use of this medium interesting data as to the types of bacteria present in the homogenizer were obtained. By using this medium it was thought that some data could also be obtained as to the types of bacteria present in the mix before and after homogenizing and also the types of bacteria present in the homogenizer.

### *Types of bacteria present*

The medium was made according to directions and the bacterial colonies counted and recorded according to the system described by Ayers and Mudge (4) on page 579 of their article, viz.:

1. A total count was made.
2. The strong acid-forming colonies were then counted. The strong acid-forming colonies are defined as, "Those with a cloudy zone about them or a slight hazy edge."
3. Weak acid-forming colonies were next counted. All colonies showing acid but without a cloudy zone or a slight hazy edge about them were considered as weak acid-forming colonies.
4. The plate was then flooded with a 5 per cent solution of acetic acid and all colonies showing the characteristic clearing about the colony were counted as peptonizers.
5. The difference between the sum of the strong acid-forming, the weak acid-forming, and the peptonizing colonies and the total count were classified as alkali formers and inert colonies.

By the use of milk-powder agar and classifying the colonies according to the above scheme, one is able to obtain a pretty good bacteriological picture of the types of colonies present.

### *Results*

The types of colonies found in the water from the homogenizer are set forth in table 8. The homogenizer was thoroughly rinsed with sterile hot water by passing several gallons through it and collecting the last part of the water and plating it on the milk-powder agar. The results show a predominance of alkaline and inert colonies with weak acid colonies next in number with nearly as many peptonizers. The strong acid-forming colonies are few in number as compared to the rest.

In table 9 are recorded the results of plating out a sample of the mix just after it had been pasteurized and before it passed through the homogenizer. The procedure followed in the College dairy is to put the materials for the mix in a starter can and pasteurize and mix it at one and the same time after which it is passed through the homogenizer. The data in table 9 are collected from samples taken as the mix is ready to pass on to the process of homogenizing.

We find a predominance of alkali and inert colonies, 42.82 per cent; with weak acid-forming colonies coming next, 34.75 per cent; with peptonizers next, 17.41 per cent; and strong acid-forming colonies last with 5.07 per cent.

TABLE 8

*Type of colonies of bacteria found in water from homogenizer*

| DATE | TOTAL COUNT | STRONG ACID | | WEAK ACID | | PEPTONIZERS | | ALKALINE AND INERT BACTERIA | |
|---|---|---|---|---|---|---|---|---|---|
| | | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent |
| *1922* | | | | | | | | | |
| 10/3 | 8 | | | | | 6 | 75.00 | 2 | 25.00 |
| 10/10 | 2,800 | 700 | 25.00 | 600 | 21.43 | 600 | 21.43 | 900 | 32.14 |
| 10/17 | 2,200 | 150 | 6.82 | 1,000 | 45.45 | 500 | 22.72 | 550 | 25.00 |
| 10/23 | 5,000 | 500 | 10.00 | 2,000 | 40.00 | 1,500 | 30.00 | 1,000 | 20.00 |
| 10/30 | 3,900 | | | 360 | 9.23 | 350 | 8.97 | 3,190 | 81.80 |
| 11/3 | 8,000 | 500 | 6.25 | 5,000 | 62.50 | 1,900 | 23.75 | 600 | 7.50 |
| 11/10 | 450 | | | 100 | 22.22 | 200 | 44.44 | 150 | 33.33 |
| 11/15 | 15 | | | | | 10 | 66.66 | 5 | 33.33 |
| 12/20 | 7,500 | | | 4,500 | 60.00 | 2,000 | 26.66 | 1,000 | 13.33 |
| *1923* | | | | | | | | | |
| 3/16 | No water saved | | | | | | | | |
| 3/21 | No water saved | | | | | | | | |
| 4/10 | 8,500 | | | | | 500 | 5.88 | 8,000 | 94.12 |
| 4/18 | 300 | 18 | 6.00 | 6 | 2.00 | 80 | 26.66 | 196 | 65.33 |
| 4/19 | 2,000 | 10 | 0.50 | | | 10 | 0.50 | 1,980 | 99.00 |
| 10/8 | 8,500 | | | 4,000 | 47.05 | 1,000 | 11.76 | 3,500 | 41.17 |
| 10/12 | 5 | | | 4 | 80.00 | 1 | 20.00 | 0 | |
| 10/19 | 5,000 | 500 | 10.00 | 3,500 | 70.00 | 800 | 16.00 | 200 | 4.00 |
| 10/25 | 4,000 | | | 500 | 12.50 | 2,500 | 62.50 | 1,000 | 25.00 |
| 11/9 | 43,000 | 3,000 | 6.97 | 18,000 | 41.86 | 10,000 | 23.25 | 12,000 | 27.90 |
| 11/13 | Spreader | | | | | | | | |
| 11/15 | 650 | | | 85 | 13.07 | 400 | 61.54 | 165 | 25.38 |
| 11/22 | 2 | | | 2 | 100.00 | | | | |
| 11/28 | 18,000 | | | 3,500 | 19.44 | 5,000 | 27.77 | 9,500 | 52.77 |
| 12/3 | 140,000 | 10,000 | 7.14 | 70,000 | 50.00 | 45,000 | 32.14 | 15,000 | 10.71 |
| *1924* | | | | | | | | | |
| 2/20 | 315 | | | 85 | 26.98 | 130 | 41.27 | 100 | 31.74 |
| Average................. | | ...... | 3.58 | | 32.90 | | 29.50 | | 34.02 |

Table 10 shows the type of colonies prevailing in the mix after it has passed through the homogenizer. It will be noticed that the difference between the number of colonies in the alkali and inert group, 36.43 per cent, and weak acid-forming group, 35.69 per cent is not so great as in table 9. It will be further noted

TABLE 9

*Type of colonies of bacteria in mix before homogenizing*

| DATE | TOTAL COUNT | STRONG ACID | | WEAK ACID | | PEPTONIZER | | ALKALINE AND INERT BACTERIA | |
|---|---|---|---|---|---|---|---|---|---|
| | | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent |
| *1922* | | | | | | | | | |
| 10/3 | 5,800 | 450 | 7.76 | 3,800 | 65.51 | 500 | 8.62 | 1,050 | 18.11 |
| 10/10 | 17,000 | 2,200 | 12.94 | 2,700 | 15.88 | 1,200 | 7.05 | 10,900 | 64.12 |
| 10/17 | 10,000* | 3,000 | 30.00 | | | 2,000 | 20.00 | 5,000 | 50.00 |
| 10/23 | 46,500 | | | 25,000 | 53.76 | 6,500 | 13.98 | 15,000 | 32.25 |
| 10/30 | 12,500* | | | 6,300 | 50.40 | 4,000 | 32.00 | 2,200 | 17.60 |
| 11/3 | 7,600* | | | 4,400 | 57.90 | 500 | 6.58 | 2,700 | 35.52 |
| 11/10 | 18,000 | | | 13,000 | 72.22 | 2,900 | 16.11 | 2,100 | 11.66 |
| 11/15 | 6,500 | 50 | 0.77 | 2,500 | 38.46 | 800 | 12.30 | 3,150 | 48.46 |
| 12/20 | 19,000 | | | 6,000 | 31.58 | 4,500 | 23.68 | 8,500 | 44.73 |
| *1923* | | | | | | | | | |
| 3/16 | 8,100 | | | 100 | 1.23 | 450 | 5.55 | 7,550 | 93.21 |
| 3/21 | 23,000 | 2,300 | 10.00 | 650 | 2.82 | 4,000 | 17.39 | 16,050 | 69.79 |
| 4/10 | 4,500 | | | | | 700 | 15.55 | 3,800 | 84.45 |
| 4/18 | 5,500 | 1,400 | 25.45 | 450 | 8.18 | 1,500 | 27.27 | 2,150 | 39.09 |
| 4/19 | 12,000 | 450 | 3.75 | 300 | 2.50 | 1,400 | 11.66 | 9,850 | 82.08 |
| 10/8 | 16,000 | 800 | 5.00 | 11,000 | 68.75 | 1,800 | 11.25 | 2,400 | 15.00 |
| 10/12 | 3,000 | 800 | 26.66 | 200 | 6.66 | 800 | 26.66 | 1,200 | 40.00 |
| 10/19 | 24,000 | | | 11,000 | 45.83 | 4,000 | 16.66 | 9,000 | 37.50 |
| 10/25 | 14,000 | | | 2,500 | 17.85 | 5,000 | 35.71 | 6,500 | 46.44 |
| 11/9 | 69,000* | | | 20,000 | 29.00 | 9,000 | 13.00 | 40,000 | 58.00 |
| 11/13 | 6,500 | | | 2,500 | 38.46 | 1,500 | 23.07 | 2,500 | 38.46 |
| 11/15 | 3,600 | | | 1,300 | 36.11 | 1,100 | 30.55 | 1,200 | 33.33 |
| 11/22 | 12,500 | | | 9,300 | 74.40 | 1,600 | 12.80 | 1,600 | 12.80 |
| 11/28 | 11,000 | 500 | 4.54 | 7,500 | 68.18 | 2,000 | 18.18 | 1,000 | 9.09 |
| 12/3 | 2,500 | | | 1,000 | 40.00 | 300 | 12.00 | 1,200 | 48.00 |
| *1924* | | | | | | | | | |
| 2/20 | 4,400 | | | 1,900 | 43.16 | 700 | 15.90 | 1,800 | 40.90 |
| Average........ | | | 5.07 | | 34.75 | | 17.41 | | 42.82 |

* Only one plate counted.

TABLE 10

*Type of colonies of bacteria in mix after homogenizing*

| DATE | TOTAL COUNT | STRONG ACID | | WEAK ACID | | PEPTONIZER | | ALKALINE AND INERT BACTERIA | |
|---|---|---|---|---|---|---|---|---|---|
| | | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent |
| *1922* | | | | | | | | | |
| 10/3 | 10,000 | 1,900 | 19.00 | 4,900 | 49.00 | 800 | 8.00 | 2,400 | 24.00 |
| 10/10 | 18,000 | 3,900 | 21.66 | 9,100 | 50.55 | 550 | 3.05 | 4,450 | 24.72 |
| 10/17 | 25,000* | 1,000 | 4.00 | 15,000 | 60.00 | 4,000 | 16.00 | 5,000 | 20.00 |
| 10/23 | 71,000 | 3,000 | 4.22 | 30,000 | 42.25 | 9,000 | 12.66 | 29,000 | 40.84 |
| 10/30 | 31,000 | 2,000 | 6.45 | 14,000 | 45.16 | 1,500 | 4.83 | 13,500 | 43.54 |
| 11/3 | 16,000* | 600 | 3.75 | 10,000 | 62.50 | 1,600 | 10.00 | 3,800 | 23.75 |
| 11/10 | 30,000 | | | 25,000 | 83.33 | 1,500 | 5.00 | 3,500 | 11.66 |
| 11/15 | 23,000 | 5,000 | 21.74 | 8,000 | 34.77 | 3,000 | 13.04 | 7,000 | 30.43 |
| 12/20 | 88,000 | 500 | 0.57 | 45,000 | 51.13 | 22,000 | 25.00 | 20,500 | 23.30 |
| *1923* | | | | | | | | | |
| 3/16 | 4,600 | 150 | 3.26 | 700 | 15.21 | 1,600 | 34.78 | 2,150 | 46.74 |
| 3/21 | 67,000 | 1,300 | 1.94 | 300 | 0.44 | 1,500 | 2.24 | 63,900 | 95.37 |
| 4/10 | 8,000 | 550 | 7.00 | | | 450 | 5.62 | 7,000 | 87.50 |
| 4/18 | 15,000 | 3,000 | 20.00 | 1,800 | 12.00 | 4,500 | 30.00 | 5,700 | 38.00 |
| 4/19 | 18,000 | 2,500 | 13.88 | 900 | 5.00 | 1,100 | 6.12 | 13,500 | 75.00 |
| 10/8 | 38,000 | 1,400 | 3.68 | 21,000 | 55.26 | 1,900 | 5.00 | 13,700 | 36.05 |
| 10/12 | 3,800 | | | 1,800 | 47.37 | 1,500 | 39.47 | 500 | 13.15 |
| 10/19 | 83,000 | 5,000 | 6.02 | 28,000 | 33.73 | 22,000 | 26.50 | 28,000 | 33.73 |
| 10/25 | 60,000* | | | 10,000 | 16.66 | 40,000 | 66.66 | 10,000 | 16.66 |
| 11/9 | 75,000* | 2,000 | 2.66 | 16,000 | 21.33 | 16,000 | 21.33 | 41,000 | 54.66 |
| 11/13 | 19,000 | 500 | 2.63 | 4,500 | 23.68 | 7,500 | 39.47 | 6,500 | 34.21 |
| 11/15 | 6,500 | | | 2,000 | 30.77 | 3,000 | 46.15 | 1,500 | 23.07 |
| 11/22 | 170,000 | 2,000 | 1.18 | 40,000 | 23.53 | 45,000 | 26.47 | 83,000 | 48.82 |
| 11/28 | 45,000 | 4,000 | 8.88 | 29,000 | 64.44 | 500 | 1.11 | 11,500 | 25.55 |
| 12/3 | 300,000 | 5,000 | 1.66 | 85,000 | 28.33 | 170,000 | 56.66 | 40,000 | 13.33 |
| *1924* | | | | | | | | | |
| 2/20 | 6,000 | | | 2,000 | 33.33 | 2,400 | 40.00 | 1,600 | 26.66 |
| Average......... | | | 6.16 | | 35.69 | | 21.81 | | 36.43 |

* Only one plate counted.

that the number of colonies of peptonizers has increased from 17.41 per cent in table 9 to 21.81 per cent in table 10 while the number of strong acid-forming colonies shows but a slight increase.

TABLE 11

*A comparison of the types of colonies before and after homogenizing*

(Plate count—milk-powder agar)

| DATE | TOTAL COUNT | | STRONG ACID | | WEAK ACID | | PEPTONIZERS | | ALKALINE AND INERT | | WATER FROM HOMOGENIZER |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After | Before | After | |
| *1922* | | | | | | | | | | | |
| 10/3 | 5,800 | 10,000 | 450 | 1,900 | 3,800 | 4,900 | 500 | 800 | 1,050 | 2,400 | 8 |
| 10/10 | 17,000 | 18,000 | 2,200 | 3,900 | 2,700 | 9,100 | 1,200 | 550 | 10,900 | 4,450 | 2,800 |
| 10/17 | 10,000* | 25,000* | 3,000 | 1,000 | | 15,000 | 2,000 | 4,000 | 5,000 | 5,000 | 2,200 |
| 10/23 | 46,500* | 71,000 | | 3,000 | 25,000 | 30,000 | 6,500 | 9,000 | 15,000 | 29,000 | 5,000 |
| 10/30 | 12,500* | 31,000 | | 2,000 | 6,300 | 14,000 | 4,000 | 1,500 | 2,200 | 13,500 | 3,900 |
| 11/3 | 7,600 | 16,000* | | 600 | 4,400 | 10,000 | 500 | 1,600 | 1,700 | 3,800 | 8,000 |
| 11/10 | 18,000 | 30,000 | | | 13,000 | 25,000 | 2,900 | 1,500 | 2,100 | 3,500 | 450 |
| 11/15 | 6,500 | 23,000 | 50 | 5,000 | 2,500 | 8,000 | 800 | 3,000 | 3,150 | 7,000 | 15 |
| 12/20 | 19,000 | 88,000 | | 500 | 6,000 | 45,000 | 4,500 | 22,000 | 8,500 | 20,500 | 7,500 |
| *1923* | | | | | | | | | | | |
| 3/16 | 8,100 | 4,600 | | 150 | 100 | 700 | 450 | 1,600 | 7,550 | 2,150 | |
| 3/21 | 23,000 | 67,000 | 2,300 | 1,300 | 650 | 300 | 4,000 | 1,500 | 16,150 | 63,900 | |
| 4/10 | 4,500 | 8,000 | | 550 | | | 700 | 450 | 3,800 | 7,000 | 8,500 |
| 4/18 | 5,500 | 15,000 | 1,400 | 3,000 | 450 | 1,800 | 1,500 | 4,500 | 1,150 | 5,700 | 300 |
| 4/19 | 12,000 | 18,000 | 450 | 2,500 | 300 | 900 | 1,400 | 1,100 | 9,850 | 13,500 | 2,000 |
| 10/8 | 16,000 | 38,000 | 800 | 1,400 | 11,000 | 21,000 | 1,800 | 1,900 | 2,400 | 13,700 | 8,500 |
| 10/12 | 3,000 | 3,800 | 800 | | 200 | 1,800 | 800 | 1,500 | 1,200 | 500 | 30 |
| 10/19 | 24,000 | 83,000 | | 5,000 | 11,000 | 28,000 | 4,000 | 22,000 | 9,000 | 28,000 | 5,000 |
| 10/25 | 14,000 | 60,000* | | | 2,500 | 10,000 | 5,000 | 40,000 | 6,500 | 10,000 | 4,000 |
| 11/9 | 69,000* | 75,000* | | 2,000 | 20,000 | 16,000 | 9,000 | 16,000 | 40,000 | 41,000 | 43,000 |
| 11/13 | 6,500 | 19,000 | | 500 | 2,500 | 4,500 | 1,500 | 7,500 | 2,500 | 6,500 | |
| 11/15 | 3,600 | 6,500 | | | 1,300 | 2,000 | 1,100 | 3,000 | 1,200 | 1,500 | 650 |
| 11/22 | 12,500 | 170,000 | | 2,000 | 9,300 | 40,000 | 1,600 | 45,000 | 1,600 | 83,000 | 2 |
| 11/28 | 11,000 | 45,000 | 500 | 4,000 | 7,500 | 29,000 | 2,000 | 500 | 1,000 | 11,500 | 18,000 |
| 12/3 | 2,500 | 300,000 | | 5,000 | 1,000 | 85,000 | 300 | 170,000 | 1,200 | 40,000 | 140,000 |
| *1924* | | | | | | | | | | | |
| 2/20 | 4,400 | 6,000 | | | 1,900 | 2,000 | 700 | 2,400 | 1,800 | 1,600 | 315 |

* Only one plate counted.

## STATISTICAL ANALYSIS OF THE SIGNIFICANCE OF CHANGES DUE TO HOMOGENIZING[2]

In table 11 is set down the total number of bacteria in the mix before and after homogenizing, also the number of each of the different types before and after the homogenizing process. This comparative data permits one to analyze statistically the significance of the changes in the number of each type before and after homogenizing.

In the computations percentages were used instead of numbers of bacteria because it was believed that this would give more accurate results.

The arithmetical mean of the differences in percentages of the different types of bacteria before and after homogenizing was determined. The standard deviation of these differences was computed and "Students" method (9) of determining the significance of the difference was used. In table 12 the results of these computations are set down.

TABLE 12

*Tabulation of the results of computation*

| TYPE | NUMBER OF EXPERIMENTS | AVERAGE DIFFERENCE | STANDARD DEVIATION OF DIFFERENCES | Z | ODDS |
|---|---|---|---|---|---|
| Strong acid | 25 | +1.93 | 8.68 | 0.2223 | 5:1 |
| Weak acid | 25 | −0.83 | 20.50 | 0.0404 | 1:1 |
| Peptonizers | 25 | +2.46 | 14.90 | 0.1650 | 2:1 |
| Alkali and inert | 25 | −6.46 | 17.20 | 0.3750 | 1:2 |

In statistical analysis odds of less than 30:1 are not considered highly significant. In the light of this fact we can not reliably state that the percentage of any type of bacteria is changed by homogenizing. This simply indicates that even though bacteria may be added to the ice cream mix from the homogenizer that the different types are added in the same relative ratio as they already existed in the mix before homogenizing. This is not hard to understand since the bacterial contamination in the homogenizer is due mostly to inadequate or improper cleaning of the machine after the mix has passed through.

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[2] The author is indebted to Prof. L. C. Emmons for his assistance in the statistical analysis of the data.

Another point brought out by the computation is the fact that during the process of homogenizing the groups of the different types are broken up in about the same relative proportion.

## GENERAL DISCUSSION

In carrying out this work an attempt was made to follow as nearly as possible factory conditions so that an actual picture would be obtained of the bacteriological changes during the process of homogenizing. In this way it was hoped to throw some light on the questions raised by the author in a previous paper and also by other workers in this field.

A close study of the data would seem to indicate that there is no definite relationship between the number of colonies on milk-powder agar and the number of bacteria found by the direct microscopic count in the ice cream mix. The type of organisms predominating in the sample might be one cause for this. If a sample had a large number of heat resistant bacteria present, a greater number would show on the plate count. A majority of the samples showed an increase in the number of colonies by the plate method after homogenizing. The same was true with the direct microscopic method. This held true both for the individuals and "groups." From a study of this data it would seem that there was some contributing cause to the increases shown.

A further study of table 2 begins to throw some light upon the cause for the increase after homogenizing. In every single case the "group" size is less. It is true that in some cases the difference is negligible. However, if one considers that the mix has been continuously agitated from forty-five minutes to an hour at a temperature of around 145°F., he will readily understand that during this agitation many of the larger clumps will have been broken up before the mix has passed through the homogenizer. That a further breaking up of clumps does occur in the homogenizer, however, is clearly indicated by the data.

Another indication that the clumps are broken up during the process of homogenizing is indicated by the total number of groups of two or more before and after hemogenizing. In 18 or 72 per cent of the samples there is a decrease, in 5 or 20 per cent of the samples there is an increase while in 2 or 8 per cent of the samples there is no change in the total number of the groups of two or more after homogenizing as compared with the total number of groups of two or more before homogenizing. When the calculations are carried still further and the average size of the groups of two or more is calculated, we again find, as in the case of the average of "groups," that there are fewer bacteria per clump after the mix has passed through the homogenizer than before. This of course indicates that there has been a breaking up of the clumps.

When milk is passed through the homogenizer the same thing holds true. There is a gradual reduction in the size of the groups as the pressure is increased. Although the reduction is slightly irregular for the few samples run yet the sum total of the results indicates that there is a reduction in the size of the groups. The results from the few samples studied—also indicate that the homogenizer is a source of contamination as shown by the increase in the counts by both methods.

A study of the types of bacteria present in the homogenizer and present before and after homogenizing presented some interesting data. It was found that the peptonizers ran considerably higher in the water from the homogenizer than they did in the mix. This no doubt accounts for at least a part of the 5.12 per cent increase of the peptonizers found in the mix after homogenizing. The only other notable change in the percentages was in the case of the alkali and inert groups where there was a decrease after as compared to before homogenizing. The strong acid-forming and the weak acid-forming types also showed an increase after homogenizing. This may have been due partly to the breaking up of clumps and partly to the contamination from the homogenizer.

However, a statistical analysis of the significance of the slight changes in the percentages of the different types before and after homogenizing shows that they are not to be taken too seriously since the odds are not sufficient to make them highly significant.

## CONCLUSIONS

1. In a majority of the cases, homogenizing increased the number of bacterial colonies in the ice cream mix as determined by the plate count using milk-powder agar.
2. In a majority of the cases, homogenizing increased the number of individuals and "groups" as determined by the direct microscopic method.
3. The average size of the "groups" and also the average size of groups of two or more in the ice cream mix are decreased. In view of the fact that all these differences are of the same sign, it is safe to conclude that homogenizing reduces the average size of bacterial groups in the ice cream mix.
4. The bacterial clumps in milk are decreased in size by passing through the homogenizer. Increasing the pressure has the general tendency to decrease the average size of the groups.
5. Milk passed through the homogenizer without pressure showed an increase in colonies by the plate method and an increase in individuals and "groups" by the direct microscopic method.
6. Sterile rinse water from the homogenizer contained from 2 to 140,000 bacterial colonies per cubic centimeter.
7. The type of colonies found in the ice cream mix were strong acid-forming, weak acid-forming, peptonizers and alkali and inert.

The general conclusion that may be drawn from the data as a whole is, that, the increase in the bacteria content after homogenizing is due to two causes: First, the breaking up of bacterial clumps and, second, contamination from the homogenizer. It would therefore, seem that a part of the increase noted in the previous papers was partly apparent and partly real.

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# A BIOMETRIC STUDY OF LENGTH OF METABOLISM EXPERIMENTS WITH CATTLE*

RAYMOND W. SWIFT

*From the Institute of Animal Nutrition, The Pennsylvania State College, State College, Pennsylvania*

A vital factor in all metabolism experiments is the irregularity in the voiding of excreta, and it is of obvious importance to have a definite expression of this irregularity as a measure of its influence on the accuracy of average figures. With a feed intake constant in amount there is a variation in the daily excretion of feces which is the result of all those factors contributing to the individuality of the subject.

This paper presents the results of a study of this variability, with cows and steers, as affecting the accuracy of metabolism experiments of different lengths. More specifically it is a comparison of the significance of figures representing average daily fecal outgo during periods of different numbers of days.

The weight of fresh feces excreted daily was used as a basis in this study, since a sufficient number of data on the dry basis were not available. The data for cows were taken chiefly from the original records of experiments performed at the Ohio Agricultural Experiment Station under the direction of Dr. E. B. Forbes, now director of this Institute. The results of these experiments are reported in Ohio Agricultural Experiment Station Bulletins Nos. 295, 308, 330, and 363. Through the courtesy of Director Williams of the Ohio Station we have been permitted to use the original records. Some additional data for cows were taken from the records of this Institute.

All data used were from periods in which the feed intake was maintained without variation and in which the intake had been at the same constant level for at least seven days previous to the first day of the collection period. These data comprise 1008 daily weights of feces from supermaintenance rations.

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*Received for publication October 14, 1924

In the study of the data a procedure similar to that of Mitchell and Grindley (1) was adopted. The weights tabulated contained five figures but it was deemed permissible and advisable to omit the last two, correcting the third for the fourth. Thus the number 27,573 was used as 276 while 26,136 was used as 261. Any error thus introduced is so slight as to be negligible.

The average figure representing the 1008 weights obtained from cow experiments was 250. Each period average was then made equal to 250 by adding to or subtracting from each weight in the period the difference between the period average and 250. As an example, in the following tabulation the number 29 is subtracted from each weight of feces in an original nineteen-day period.

| ORIGINAL DATA | TRANSMUTED |
|---|---|
| 279 | 250 |
| 261 | 232 |
| 276 | 247 |
| 294 | 265 |
| 249 | 220 |
| 280 | 251 |
| 284 | 255 |
| 272 | 243 |
| 269 | 240 |
| 297 | 268 |
| 276 | 247 |
| 294 | 265 |
| 289 | 260 |
| 296 | 267 |
| 283 | 254 |
| 288 | 259 |
| 264 | 235 |
| 280 | 251 |
| 275 | 246 |
| Average......279 | 250 |

It is clear that neither the individual deviations from the respective means nor consequently the standard deviation, has been changed. The object of this transmutation was to make every weight deviate from the same average, namely 250, thus

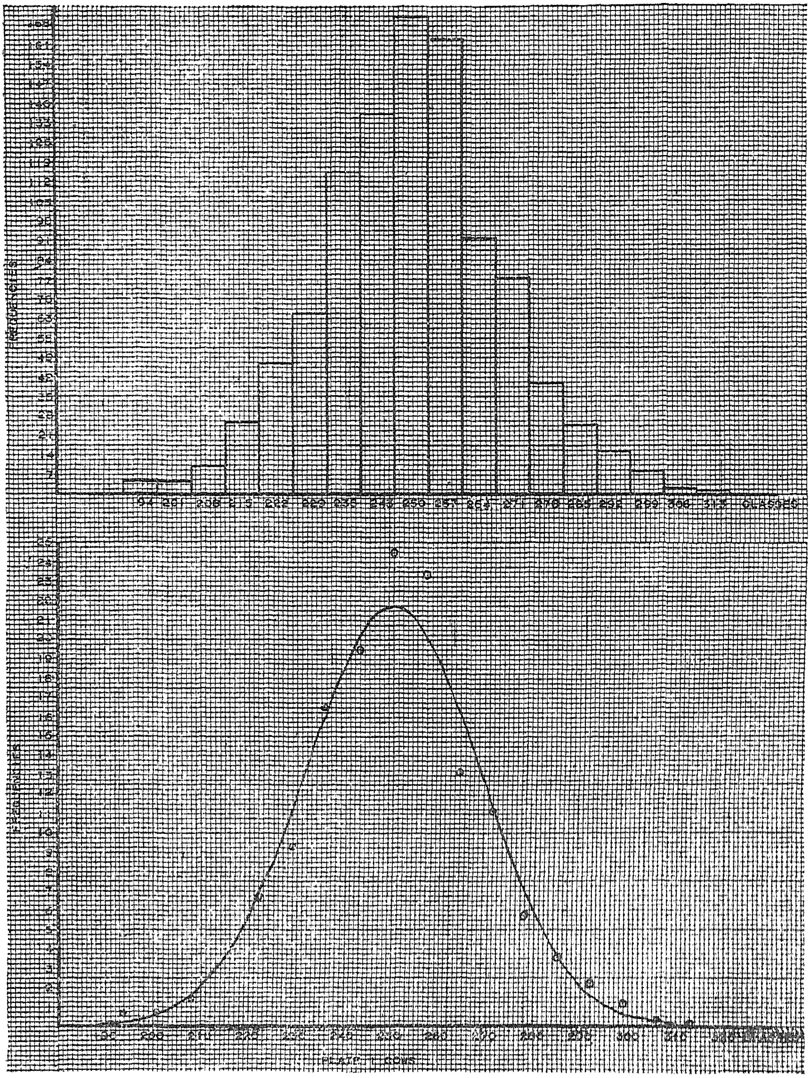

FREQUENCIES
94
201
208
215
222
229
236
243
250
257
264
271
278
285
292
299
306
313
CLASSES
FREQUENCIES
200
300
PLATE 1 COWS

PLATE 1

eliminating the effect of the plane of feed intake on the average weight of feces for each individual period. These transmuted weights were then arranged arbitrarily, and the standard deviation from the above stated average (250) of the 1008 weights was found to be 19.1. In testing the suspicion that a few very widely divergent individual weights should be rejected, a limiting deviation was calculated according to Chauvenet's criterion (2). The weights thus rejected were respectively 176, 324, 180, 169, and 319, the limiting range being 183 to 317. This elimination changed the grand total average so slightly that it was unnecessary to determine a new one. The standard deviation of the remaining 1003 weights was 18.4.

The frequency distribution of the entire lot of weights is shown in the upper part of plate 1. This series of rectangles illustrates the actual frequency distribution of the 1003 weights. The curve drawn in the lower part of the graph is the normal frequency curve derived from the equation

$$y = \frac{n}{\sigma\sqrt{2\,\pi}} \times \frac{1}{e^{\frac{x^2}{2\,\sigma^2}}}$$

The closeness of the actual distribution to the normal curve is shown by the points enclosed by circles which are taken directly from the center points of the tops of the rectangles above. This closeness of fit is quite satisfactory.

The 1003 weights were then averaged by groups of 8, 12, 16, 20 and 24. Thus there were 125 values for eight-day period averages, 62 for sixteen-day periods and so on, there being 41 values for twenty-four-day period averages. Treating the different averages determined as variates, the standard deviation of the 125 eight-day period averages was found to be 6.42, that of the 83 twelve-day period averages 5.05, and for sixteen-, twenty-, and twenty-four-day periods the values were 3.89, 3.35, and 2.44, respectively. The average of all weights for periods of a given length was of course 250, so that the figure 6.42 represents the standard deviation of eight-day period averages around the mean 250. Collection periods of at least eight days were assumed to be necessary for satisfactory results while twenty-four days was assumed as a practical maximum.

The following tables show the results obtained and an interpretation of them in reference to their probable errors, etc.

Taking ±4.33 as the probable error of a single eight-day period average and multiplying it by 3.2 we obtain a limiting value (right-hand column) such that the odds are 31 to 1 that any one of the eight-day period averages will fall within this range. This represents the manner in which the limiting values for all the periods have been obtained. In table 2 the values have been expressed in percentage.

TABLE 1

*Variability in period-average weights of cow feces*

| LENGTH OF PERIOD | NUMBER OF VARIATES (PERIOD AVERAGES USED) | STANDARD DEVIATION | PROBABLE ERROR OF A SINGLE VARIATE (A PERIOD AVERAGE) 0.6745 × ST. DEV. ($E_S$) | LIMIT OF ERROR; ODDS 31 TO 1 ($E_S$ × 3.2) |
|---|---|---|---|---|
| *days* | | | | |
| 8 | 125 | 6.42 | ±4.33 | ±13.86 |
| 12 | 83 | 5.05 | ±3.41 | ±10.91 |
| 16 | 62 | 3.89 | ±2.62 | ±8.38 |
| 20 | 50 | 3.35 | ±2.26 | ±7.23 |
| 24 | 41 | 2.44 | ±1.65 | ±5.28 |

TABLE 2

*Derivation of percentage statement of error in period-average weights of cow feces*

| LENGTH OF PERIOD | AVERAGE | LIMIT OF ERROR; ODDS 31 TO 1 | RANGE OF CERTAINTY (31 TO 1) | LIMIT OF ERROR: IN PER CENT OF MEAN (ODDS 31 TO 1) |
|---|---|---|---|---|
| *days* | | | | |
| 8 | 250 | ±13.86 | 236.14 to 263.86 | 5.54 |
| 12 | 250 | ±10.91 | 239.09 to 260.91 | 4.36 |
| 16 | 250 | ±8.38 | 241.62 to 258.38 | 3.35 |
| 20 | 250 | ±7.23 | 242.77 to 257.23 | 2.89 |
| 24 | 250 | ±5.28 | 244.72 to 255.28 | 2.11 |

Odds of 31 to 1 have been chosen arbitrarily as being sufficient to warrant the conclusion that when these odds exist we may be practically certain that any single variate chosen at random will fall within these limits. This limit of error, with odds 31 to 1, stated in per cent of the mean, is computed from the limit of error in absolute terms by dividing by 250 and multiplying by 100. These percentage expressions signify the reliability of eight-, twelve-, sixteen-, twenty- and twenty-four day period averages. Expressed in words this means that the average daily fecal outgo as measured in an eight-day experiment with cows on a supermaintenance ration is not more than 5.54 per cent in error. In the case of a twenty-four-day collection period this error is not greater than 2.11 per cent.

Plate 3 illustrates graphically the different distributions of the averages of the various lengths of periods. Each curve here shown is the normal curve based on the number of variates and the standard deviation. As there were approximately three times as many eight-day periods as twenty-four-day periods the units on the ordinate were made in proportion to these lengths of periods so that each curve would enclose about the same area since it is desirable to depict the form of the curves alone. The normal curve should never quite touch the abscissa.

It was thought that there being 125 variates for eight-day period values and only 41 for twenty-four-day periods would perhaps tend toward an unfair comparison. Consequently the 125 variates were listed on pieces of cardboard, thoroughly shuffled, and 41 were drawn at random. The standard deviation of these 41 variates was found to be 5.71, with a probable error of ±0.43. The standard deviation of the 125 variates was 6.42 ± 0.27. Since the difference between these two standard deviations is less than twice the square root of the sum of the squares of their respective probable errors, the difference is insignificant.

The data concerning steer experiments were used in a manner similar to that outlined for cows, and were taken from experiments conducted at the Pennsylvania Experiment Station and the Institute of Animal Nutrition and reported in Bulletin No. 42, United States Department of Agriculture, Bureau of Animal Industry Bulletin Nos. 51, 74, 101, and 128. In these experiments the animals were given constant amounts of feed but the plane of intake was very close to the maintenance requirement, the variability in the elimination of feces, therefore, being characteristic of this restricted intake rather than of full feeding. The 960 weights thus obtained were treated as were the 1008 weights relating to cows, the average of the 960 being 100. These were transmuted in the manner described for the cow experiments, thus making all original period averages equal to 100. The standard deviation from the average (100) of the 960 weights was 11.75. Five weights were rejected by the method above referred to, namely, 143, 34, 153, 47, and 142, the limiting range being 59 to 141. The rejection of these weights did not significantly change the average of the remaining 955. The standard deviation of these 955 weights was 11.17. The actual frequency

TABLE 3

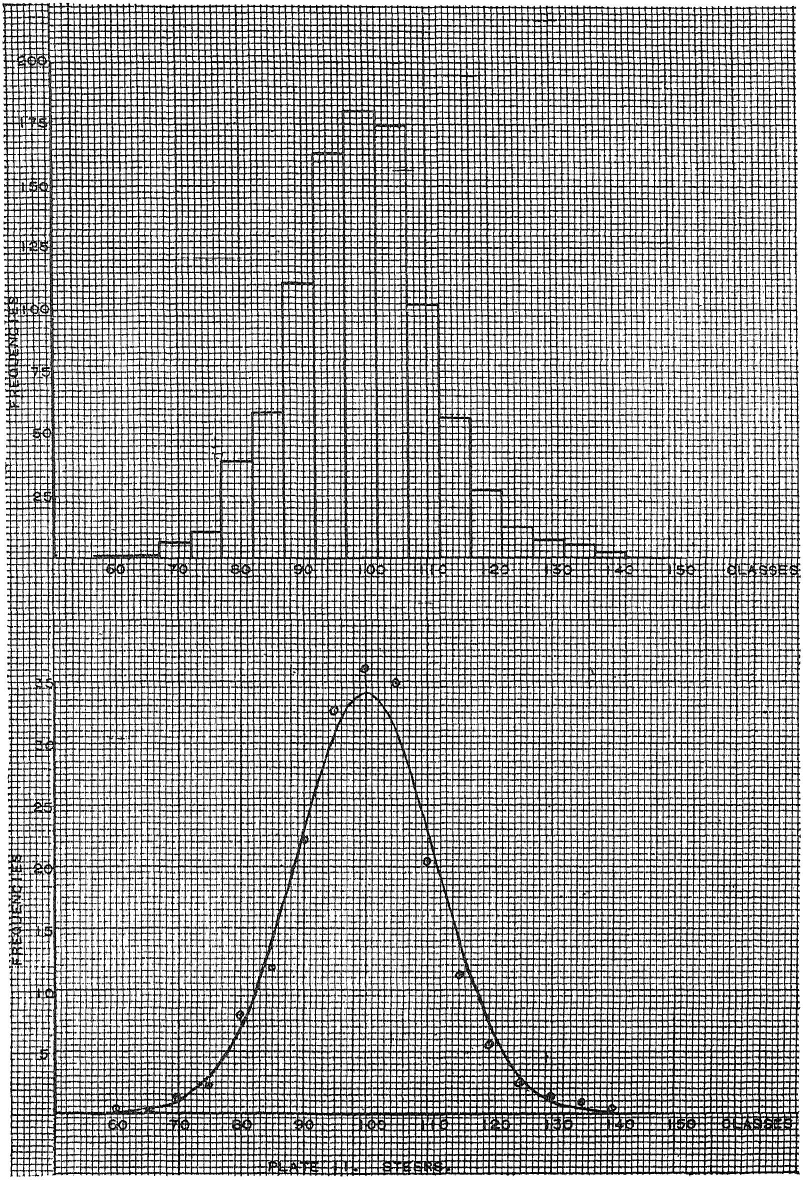
*Variability in period-average weights of steer feces from maintenance experiments*

| LENGTH OF PERIOD | NUMBER OF VARIATES (PERIOD AVERAGES USED) | STANDARD DEVIATION | PROBABLE ERROR OF A SINGLE VARIATE (A PERIOD AVERAGE) 0.6745 × ST. DEV. ($E_S$) | LIMIT OF ERROR ODDS 31 TO 1 ($E_S$ × 3.2) |
|---|---|---|---|---|
| *days* | | | | |
| 8 | 119 | 3.36 | ±2.27 | ±7.26 |
| 12 | 79 | 2.81 | ±1.90 | ±6.08 |
| 16 | 59 | 2.43 | ±1.64 | ±5.25 |
| 20 | 47 | 2.12 | ±1.43 | ±4.58 |
| 24 | 39 | 1.81 | ±1.22 | ±3.90 |

TABLE 4

*Derivation of percentage statement of limit of error in period-average weights of steer feces from maintenance experiments*

| LENGTH OF PERIOD | AVERAGE | LIMIT OF ERROR; ODDS 31 TO 1 | RANGE OF CERTAINTY (31 TO 1) | LIMIT OF ERROR; IN PER CENT OF MEAN (ODDS 31 TO 1) |
|---|---|---|---|---|
| *days* | | | | |
| 8 | 100 | ±7.26 | 92.74 to 107.26 | 7.26 |
| 12 | 100 | ±6.08 | 93.92 to 106.08 | 6.08 |
| 16 | 100 | ±5.25 | 94.75 to 105.25 | 5.25 |
| 20 | 100 | ±4.58 | 95.42 to 104.58 | 4.58 |
| 24 | 100 | ±3.90 | 96.10 to 103.90 | 3.90 |


200
175
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125
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50
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FREQUENCIES
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80
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CLASSES
35
30
25
20
15
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FREQUENCIES
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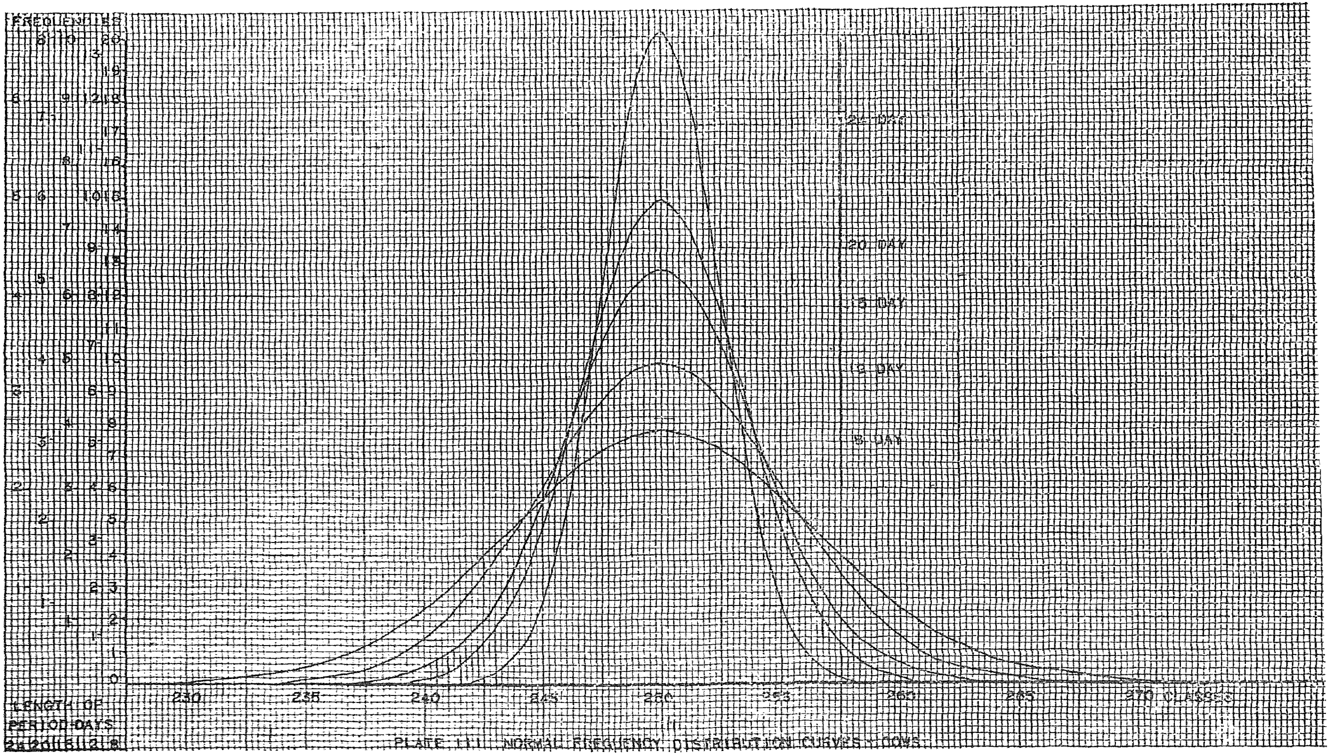
CLASSES
PLATE 11. STEERS.

PLATE 2


FREQUENCIES
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3
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11
12
13
14
15
16
17
18
19
20
LENGTH OF
PERIOD DAYS
24 20 15 12 8
230
235
240
245
250
255
260
265
270 CLASSES
24 DAY
20 DAY
15 DAY
12 DAY
8 DAY
PLATE III NORMAL FREQUENCY DISTRIBUTION CURVES - COWS

Plate 3

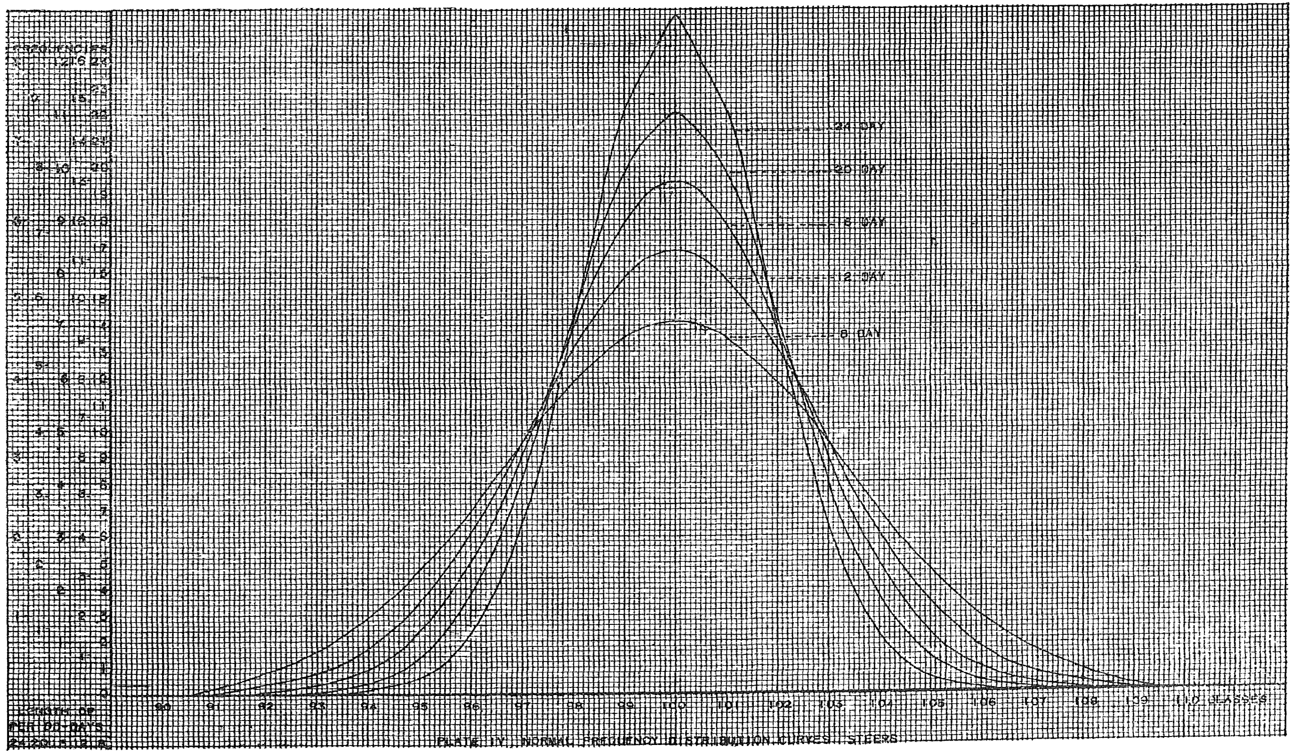
FREQUENCIES
LENGTH OF
PERIOD-DAYS
24 20 16 12 8
0
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110 CLASSES
24 DAY
20 DAY
16 DAY
12 DAY
8 DAY
PLATE IV. NORMAL FREQUENCY DISTRIBUTION CURVES. STEERS

PLATE 4

distribution of the entire lot, as compared with the normal curve, is shown in plate 2.

The weights were then averaged by groups of 8, 12, 16, 20, and 24, as in the case of the cows, with the results shown in table 3. The standard deviation of the 119 eight-day period averages was 3.36. A second arrangement by eights gave a standard deviation of 3.59, the difference between this and the above value being insignificant.

As the mean in this case was 100, the same values will appear in table 4 in which the limit of error is stated in per cent. Thus 100 ± 7.26 means of course 100 ± 7.26 per cent. Plate 4 illustrates graphically the normal frequency distributions based on the above values.

TABLE 5

*Percentage statement of limits of error in period-average weights of cow and steer feces*

| LENGTH OF PERIOD | STEERS | COWS |
|---|---|---|
| *days* | | |
| 8 | 7.26 | 5.54 |
| 12 | 6.08 | 4.36 |
| 16 | 5.25 | 3.35 |
| 20 | 4.58 | 2.89 |
| 24 | 3.90 | 2.11 |

It will be seen from a comparison of tables 2 and 4 that the data for steers give a higher variability than those for cows, doubtless due to the marked difference in planes of nutrition prevailing in the experiments. Thus the limit of error of the average weight of feces from a sixteen-day period experiment on steers which received approximately a maintenance ration is but little smaller than the corresponding value from an eight-day experiment on cows which received a full feed. For convenience the limits of error, in per cent of the mean, from both the cow and the steer experiments are assembled in table 5.

It should be remembered that these percentages do not represent the probable errors of these lengths of periods, but signify rather that the odds are 31 to 1 that the percentage errors will not be in excess of these values. They express the degree of reliability of the period-average feces weights from experiments with cows on full feed and steers on an approximate maintenance basis.

## SUMMARY

From a statistical study of 1008 weights of cow feces, and 960 weights of steer feces, resulting from metabolism experiments of varying length but constant feed intake, it is shown that with cows the chances are 31 to 1 that with collection periods varying in length from eight days to twenty-four days the errors of the averages will not exceed 5.54 to 2.11 per cent; and that with steers on restricted feed intake, the chances are, similarly, that with collection periods of eight to twenty-four days the errors of the averages will not exceed 7.26 to 3.90 per cent.

The experimenter will be his own judge as to the degree of variability permissible in a particular piece of research.

## ACKNOWLEDGMENTS

I wish to express my thanks to Director E. B. Forbes of this Institute for suggesting this problem to me, to Director C. G. Williams, of the Ohio Agricultural Experiment Station for the courtesy of the loan of original records, and to W. W. Braman for helpful suggestions throughout this study.

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# A COMPARISON OF THE METHYLENE BLUE REDUCTASE TEST AND THE DIRECT MICROSCOPIC COUNT IN GRADING MILK AT MILK PLANTS*

H. C. TROY

*Department of Dairy Industry, Cornell University, Ithaca, New York*

During recent years the methylene blue reductase test has been advocated as a practical method to use for determining the approximate number of bacteria in milk. The test has been used quite extensively in some European countries and recently to some extent in this country at dairy manufacturing plants and milk receiving stations.

The well established plate count method and the more recent direct microscopic count method have been adopted as official by the American Public Health Association. These tests are more expensive and complicated and demand more care and skill than is required in operating the reductase test. But the latter test has not been used in this country to such an extent that its place in the dairy industry has been definitely established.

During the months of July, August, and September, 1924, a number of dairy manufacturing plants and milk receiving stations were visited by the writer and a sample of the milk delivered by each producer was tested for bacteria by both the methylene blue reductase test and the direct microscopic method. The object of the work was first, to determine the grade (A, B or C) of each producer's milk on the day it was tested and, second, to learn how the results obtained by the reductase test checked with those obtained by the direct microscopic count method.

## METHOD OF PROCEDURE

The operator of the tests visited each plant once and remained there from two days to one week, the time varying according to the number of samples tested. The apparatus used in making

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*Received for publication January 24, 1925.

the reductase test was an outfit obtained from a dairy supply concern. In some instances samples of the night's milk only were tested. In others, samples of both the night's and morning's milk were taken and tested separately; but the usual practice was to take a small amount of the milk from each can of the night's and morning's milk that a producer delivered in the morning and place these different portions together in a sterile bottle to serve as a sample of his milk. The samples, usually, were iced in a tray when taken. When the desired number were obtained the tray of samples was taken to the laboratory in the plant.

TABLE 1

*Summary of data in the comparison of the methylene blue reductase test and the direct microscopic count in grading milk at milk plants*

| PLANT NUMBER | NUMBER OF SAMPLES | REDUCTASE TEST | | | MICROSCOPIC COUNT | | | NUMBER IN SAME GRADE BY BOTH METHODS | NUMBER PLACED IN DIFFERENT GRADES | NUMBER IN A LOWER GRADE BY REDUCTASE TEST | NUMBER IN A LOWER GRADE BY MICROSCOPIC COUNT | PERCENTAGE AGREEMENT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Grade A | Grade B | Grade C | Grade A | Grade B | Grade C | | | | | |
| 1 | 94 | 71 | 9 | 14 | 60 | 18 | 16 | 68 | 26 | 10 | 16 | 72.2 |
| 2 | 87 | 67 | 10 | 10 | 59 | 15 | 13 | 68 | 19 | 4 | 15 | 78.1 |
| 3 | 68 | 44 | 4 | 20 | 36 | 8 | 24 | 58 | 10 | 0 | 10 | 85.3 |
| 4 | 95 | 44 | 15 | 36 | 44 | 19 | 32 | 83 | 12 | 4 | 8 | 87.3 |
| 5 | 200 | 135 | 24 | 41 | 136 | 23 | 41 | 170 | 30 | 12 | 18 | 85.0 |
| 6 | 100 | 72 | 13 | 15 | 72 | 12 | 16 | 82 | 18 | 8 | 10 | 82.0 |
| 7 | 225 | 122 | 20 | 83 | 125 | 35 | 65 | 182 | 43 | 24 | 19 | 80.8 |
| 8 | 100 | 72 | 11 | 17 | 72 | 10 | 18 | 93 | 7 | 3 | 4 | 93.0 |
| 9 | 100 | 80 | 6 | 14 | 78 | 6 | 16 | 86 | 14 | 5 | 9 | 86.0 |
| 10 | 100 | 86 | 7 | 7 | 83 | 12 | 5 | 87 | 13 | 1 | 12 | 87.0 |
| 11 | 100 | 72 | 8 | 20 | 65 | 14 | 21 | 85 | 15 | 3 | 12 | 85.0 |
| 12 | 100 | 83 | 6 | 11 | 74 | 14 | 12 | 89 | 11 | 1 | 10 | 89.0 |
| 13 | 98 | 84 | 4 | 10 | 84 | 6 | 8 | 94 | 4 | 3 | 1 | 96.0 |
| 14 | 96 | 80 | 5 | 11 | 72 | 11 | 13 | 86 | 10 | 1 | 9 | 89.5 |
| 15 | 50 | 47 | 1 | 2 | 42 | 6 | 2 | 46 | 4 | 0 | 4 | 92.0 |
| 16 | 84 | 80 | 4 | 0 | 77 | 5 | 2 | 79 | 5 | 0 | 5 | 94.0 |
| 17 | 100 | 80 | 14 | 6 | 73 | 18 | 9 | 88 | 12 | 1 | 11 | 88.0 |
| 18 | 150 | 125 | 6 | 19 | 118 | 13 | 19 | 137 | 13 | 4 | 9 | 91.3 |
| Totals.... | 1,947 | 1,444 | 167 | 336 | 1,370 | 245 | 332 | 1,681 | 266 | 84 | 182 | 86.3 |

The reductase test and the direct microscopic count were made as soon after the samples were obtained and as rapidly as the work could be done conveniently.

In the reductase test the tubes in the incubator were held in a water bath at 100° to 105°F., usually 103°F. and were examined at the end of the first hour and at half-hour intervals thereafter up to five hours. When the time required to reduce the methylene blue color was more than three hours, the milk was classed as Grade A. When the time was three hours or more than two hours, it was classed as Grade B. When the time was two hours or less, it was classed as Grade C. This basis of grading was advised by the firm that placed on the market the apparatus for making the test. The concentration of methylene blue solution supplied with the apparatus was such that it gave 1 part of the dry crystalline dye to 200,000 parts of milk in the test.

In making the direct microscopic count single bacteria and clusters were each counted as one source. But when a smear showed few single bacteria or pairs and relatively large numbers of clusters some allowance was made for the larger number of bacteria in the clusters.

All samples of milk containing less than 300,000 sources per cubic centimeter by the direct microscopic count were classed as Grade A. Samples containing 300,000 sources and upwards to 1,500,000 were classed as Grade B. All samples that contained 1,500,000 bacteria per cubic centimeter or over that number were classed as Grade C.

The correlation between the two methods was 86.2 per cent. It was highest at the plants that received the largest proportions of milk of Grade A quality.

The microscopic count placed more samples in lower grades than were so placed by the reductase test. There were 266 samples, out of 1947 samples tested, for which the methods did not agree as to the Grade in which the milk should be placed. Of these 266 samples, 182 were placed in a lower grade by the direct microscopic count method, whereas, only 84 samples were placed in a lower grade by the reductase test.

In those instances where the two tests did not agree, the milk, usually, was placed in adjoining grades, but there were a few instances where milk was placed in the highest grade by one method and in the lowest grade by the other. Some of these instances may have been caused by errors in technique but probably not all of them.

# DAIRY NOTES

## MORE HIGH GRADE ARTICLES NEEDED

Nearly all manuscripts approved by the editorial board have now been published in the JOURNAL OF DAIRY SCIENCE. From this time on we hope to be able to publish articles shortly after they have gone through the regular channels.

The editorial board is very anxious to give space only to high grade articles and to preserve a happy balance of material of interest to the production side as well as those interested largely in the manufacturing divisions of the dairy industry.

May I not urge that all persons having available manuscripts forward them to the editor at once. Help us to improve the scope and quality of the JOURNAL by sending us your *best* papers *now* while the material is up to date and timely.

J. H. FRANDSEN,
*Editor.*

## DAIRY SCIENCE NOTES

The headquarters for the Dairy Science Association during the National Dairy Exposition at Indianapolis will be the Hotel Severin. This hotel is reserving a block of rooms for the members of the association and those of you interested in obtaining a reservation should do so at once. These rooms will be held for us a short time after which those not filled will be cancelled. The best rates that could be obtained at this or any other hotel was from $5 to $7 per double room, all rooms with bath. In writing to this hotel mention the fact that you are a member of the American Dairy Science Association.

The following men have been designated as a committee in the Intercollegiate Dairy Cattle Judging Contest:

W. W. Swett, *Chairman*
H. H. Kildee
A. A. Borland
L. H. Fairchild
L. W. Ingham
J. W. Fuller
William Regan
J. B. Fitch

The members of committees of Section III, the Extension section, are as follows:

*Dairy Manufacturing*
- J. D. Brew, Ithaca, N. Y.
- A. W. Rudnick, Ames, Iowa
- G. N. Tobey, Knoxville, Tenn.

*Feeding*
- E. B. Fitts, State College, Pa.
- C. B. Finley, Ames, Iowa
- W. J. Keegan, Clemson College, S. C.
- A. R. Morrill, Storrs, Conn.
- H. A. Hopper, Ithaca, N. Y.

*Milk Campaigns*
- Jessie M. Hoover, U. S. Bureau of Dairying, Washington, D. C.
- W. P. B. Lockwood, Amherst, Mass.
- Dorothy S. Bulkley, Storrs, Conn.
- Mary E. Thomas, Baton Rouge, La.

*Bull Associations*
- L. A. Higgins, Starkville, Miss.
- G. G. Cushman, Clemson College, S. C.
- M. J. Regan, Columbia, Mo.
- A. R. Merill, Storrs, Conn.

*Calf Clubs*
- E. J. Perry, New Brunswick, N. J.
- E. M. Harmon, Columbia, Mo.
- C. A. Hutton, Knoxville, Tenn.
- R. H. Olmstead, State College, Pa.

*Cow Testing Associations*
- A. C. Baltzer, East Lansing, Mich.
- A. J. Cramer, Madison, Wis.
- E. A. Hanson, St. Paul, Minn.
- C. R. Gearhart, State College, Pa.
- L. P. Emmerick, Blacksburg, Va.

J. B. Fitch,
*Secretary.*

## HYDROCYANIC ACID RETAINED BY FUMIGATED CHEESE

Messrs. E. R. de Ong and C. L. Roadhouse have recently published the recommendation that cheese may be fumigated with hydrocyanic acid gas for the destruction of certain cheese pests ("Cheese Pests and Their Control," Calif. Exp. Sta. Bul. 343, May, 1922). The ability to use this gas with safety in cheese rooms without removing the cheese is much to be desired and in connection with his work with the cheese skipper (Piophila casei L.) the writer made several preliminary tests of the advisability of using this fumigant. He is indebted to Dr. R. E. Holm, division of Virus-Serum Control, Bureau of Animal Husbandry, U. S. D. A., for the analyses of the fumigated cheese. A review of the experiments and results follows.

October 10–11, 1921. Fumigating box, 100 cubic feet. Charge 4 ounces NaCN, 6 ounces $H_2SO_4$, 8 ounces water. Temperature at start about 55°F.; relative humidity about 80 per cent. Exposure of cheese 10:30 a.m. to 8:45 a.m. Cheese tested: Camembert, Cheddar and Swiss, the pieces being roughly 3 by 3 by 3 inches. One side of each piece freshly cut and marked. Gas not strong when box opened. Analysis showed considerable HCN in the cheese, especially in the Camembert; least in the Cheddar. The gas in the cheese much reduced after airing about one day.

October 17–18, 1921. Fumigating box the same, but repaired to reduce leakage. Charge 2 ounces NaCN, 3 ounces $H_2SO_4$, 4 ounces water. Temperature about 63°F.; relative humidity 89 per cent. Exposure of cheese 3:00 p.m. to 9:30 a.m. Gas strong when box opened. Cheese tested, same as in first trial. Analyses showed that a large amount of acid was retained, even after airing for about one day. After this airing, more acid was present in the center of the cheese near the surface, due probably, to slower dissipation from within.

October 18–19, 1921. Fumigating box the same. Charge 1 ounce NaCN; 1½ ounces $H_2SO_4$, 2 ounces water. Temperature about 65°F.; relative humidity 80 per cent. Exposure of cheese 11:30 a.m. to 10:00 a.m. Temperature steadily declined to 55°F. at end of trial. Migrant skipper larvae used as indicators of efficiency of the fumigation were killed; these were exposed in Petri dishes. Cheese aired for 24 hours before being sent to Dr. Holm's laboratory. Analyses showed that HCN was found in all the samples, but the concentration in the Cheddar amounted to a mere trace.

On account of the tenacity with which skipper larvae cling to life in the presence of hydrocyanic acid gas, especially when embedded in their food materials, the writer feels that dosages up to and including one ounce per 100 cubic feet would be largely wasted. Cheese mites also are reported to be very resistant to this gas. It would not be safe to assume that all cheeses in a given room were protected by an unbroken coating of paraffin, and in view of the very evident tendency of cheese to retain this poisonous acid and the fact that cheese is not usually cooked before being eaten (a process which would be expected to aid the volatilization of the retained acid), the writer feels that until more extensive tests are made to prove otherwise, in retard to each variety of cheese, cyanide fumigation of cheese should not be recommended.

PEREZ SIMMONS,
*Entomologist, Stored Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture.*

## FOURTH ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

*Reported by J. A. Gamble*

The meeting was called to order by the Chairman, J. P. LaMaster, at Atlanta, Ga., February, 1925, with 21 Southern Dairy workers present.

Professor LaMaster, in his presidential address, covered the developments in dairy husbandry in the Southern States during the past four years and made suggestions for the guidance of the meeting.

The first speaker was J. H. McClain of the United States Dairy Bureau who outlined the Projects in Dairy Husbandry which should have the attention of the dairy workers in the different parts of the South and made some suggestions for inaugurating and carrying forward this work. These suggestions were discussed by Mr. Oliver of the American Jersey Cattle Club, Mr. Humphries of Georgia, J. S. Moore of Mississippi, and V. M. Williams of North Carolina.

Professor Wylie delivered an outstanding address supported by charts on "The Age, Season and Lactation Factors in Milk Production." This was one of the outstanding contributions to the meeting.

Mr. Oliver of the American Jersey Cattle Club addressed the convention advocating the formation of more Boy's Calf Clubs and the necessity of starting new breeders right.

Mr. Stanley Combs of the Holstein Friesian Association gave an inspiring talk on the "Benefits resulting from the Introduction of Pure Bred Cattle into the South" and made a special plea for the formation of Cow Testing Associations, Bull Associations and Calf Clubs. He made the point that in bringing better dairying into the South, we must reach the man with a few cows. He stated that he was working for the advancement of dairying in the South first, knowing that as a result of such work the Holstein Friesian would come into her proper place.

Mr. Humphries of the Southern Railroad was called on and gave a splendid talk relating to the Need for Increasing the Production of Cows and Increasing the Care in Maintaining Quality, suggesting Cow Testing Associations and Bull Associations as a means for this end. He further suggested that the dairy workers in each of the Southern States come together at least once a year and discuss their State problems.

Mr. McWorter of the Georgia Railroad discussed the work his road was doing in bringing about improved pastures in the State of Georgia and the allied work which has been carried forward continuously by his Company for the past several years.

L. C. Wells of the Experiment Station, Alabama, in discussing his dairy problems said that their effort was to get a few dairy farms on a profitable basis in Alabama where better feeding and better breeding could be pointed to as an example for others to follow. He was of the opinion that such men would do more to place dairying on a foundation from which it can grow prominently then efforts less enduring.

Mr. McCracken of the L & N Railroad discussed the interest of his company in Calf Club Work and suggested that they might be induced to support the annual Dairy Cattle Judging Contest held at the Tri-state Fair, Memphis.

The report of the Dairy Cattle Judging Contest, submitted by Chairman J. J. Hooper was read by the Secretary and discussed by several members of the committee. It was the concensus of opinion that the Tri-state Judging Contest should be supported by all members of the Southern Division of the American Dairy Science Association.

Mr. Wylie suggested that the Chairman, J. J. Hooper, get in touch with the teachers in Dairy Cattle Judging early in the spring and continue to push the interest in the affair urging also that Kentucky be represented. The report of Chairman Hooper was accepted with thanks and the committee continued.

The report of the Resolution Committee was read and adopted.

### *Resolution of the Dairy Husbandry Section*

The Committee on resolutions for the Southern Division of the American Dairy Science Association recommend the following:

I. That we urge the active efforts of all agencies interested in Southern Agriculture toward the furtherance of the eradication and control of diseases detrimental to the progress of the dairy industry, some of the more important being tuberculosis and contagious abortion. Especial effort is urged toward the task of completing the eradication of the cattle tick.

II. That we strongly urge the greater consumption of dairy products from the standpoint of growth and development of the young and proper nourishment of all, the fostering of efforts now being put forth toward this end is solicited.

III. In view of the progress dairy farming has made during the past few years of stress in the cotton belt and in view of the further fact that there are great possibilities for development in this industry in the South, we ask a careful study of the relation that the dairy industry should bear to a well rounded system of agriculture to the end that all interested in Southern Agricultural development may lend a hand in a unified way in the furtherance of such a system.

IV. That the active and closest coöperation of the agronomy workers of the South is needed and earnestly solicited in behalf of crops and pastures best suited for producing feed for dairy cattle. Without the basic principle of cheap home grown feed dairying cannot reach its most profitable development and help is needed to establish the proper cropping systems for various soil types in the South.

V. That the Southern Division of the American Dairy Science Association wishes to express its appreciation and thanks for the aid of public spirited organizations in the support of constructive projects for the development of dairying in the South. We urge unified effort in the formation and execution of policies for further work in behalf of the industry.

### *Second session*

The Extension Committee report was presented by C. A. Hutton, was discussed at length and adopted.

L. S. Edwards of Tennessee delivered the report of the Dairy Manufacturing and Marketing Committee. This showed splendid work and was much appreciated by those present. The report was adopted.

Report on Official Testing, by J. S. Moore of Mississippi was listened to with a great deal of interest, discussed fully and adopted.

The report of the Nominating Committee was next received and adopted as follows:

Chairman: C. A. Hutton, Tennessee; J. S. Moore, Mississippi.

Vice-Chairman: J. A. Gamble, Maryland; F. W. Fitch, Georgia.

Secretary-Treasurer: J. P. LaMaster, South Carolina; J. S. Arey, North Carolina.

In the absence of A. C. Baer, the Secretary read his paper on "Southern Dairy Manufacturing."

The report of progress dealing with the work of the Southern Livestock Research Board was read by V. M. Williams of North Carolina.

J. A. Gamble discussed the work of the College Feed Conference Board and called attention to the fact that the next meeting was to be held in New York on February 27th and 28th suggesting that it might be of interest to several present.

The report on Animal Breeding by Dr. Lush was read by the Secretary.

Professor Barre, Secretary of the Association of Agricultural Workers being present was introduced and after complimentary remarks asked that all of the papers delivered before the Convention be abstracted so that they might be printed among other section material in the annual report of the Association of Southern Agricultural Workers.

J. A. Gamble reported the latest developments relative to changes in the constitution of the American Dairy Science Association which would permit the formation and affiliation of Junior Dairy Clubs.

# THE MINERAL METABOLISM OF DAIRY COWS AS AFFECTED BY DISTILLED WATER AND PREVIOUS FEEDING*

C. F. MONROE AND A. E. PERKINS
*Ohio Agricultural Experiment Station, Wooster, Ohio*

The results of our former mineral metabolism experiments (1) have raised some questions in our minds. These results differed from those of other investigators, principally, in that our cows showed a better utilization of calcium. We failed to find any sensational negative balances for this element which some have believed were inevitable with cows producing as these did, 30 pounds or more of milk per day.

We were reluctant to think, however, that our results were inconsistent with the history of the animals. In our previous discussion (1) we summarized the situation as follows:

> As a matter of fact, we are convinced, after examining their milk records in the several lactation periods, that the assimilation of calcium by these cows must have been more favorable than most of the mineral metabolism experiments have shown; for, if this had not been true, either the milk production would have been considerably less or the animals themselves would have come to disaster.

## COMPARISON OF RESULTS

In comparing our experiments with others relating to this same subject we were impressed with certain factors which may have caused the more favorable balances for our cows. These factors are briefly discussed in the following paragraphs.

### 1. *Vitamin content of hay*

The quality of the clover hay fed in our experiments deserves consideration, especially in view of the evidence submitted by

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* Published with the approval of the Director of the Ohio Agricultural Experiment Station. Received for publication April 10, 1925.

Hart and associates (2). These workers observed more favorable calcium balances when (3) feeding alfalfa hay cured under caps than when feeding (4) alfalfa hay cured by four days' exposure to the sun and weather while in the window. They suggest that curing hay without undue exposure to the sun and weather aids in preserving the unknown factors affecting calcium assimilation (3). The clover hay used in our experiments was of choice quality, and had retained remarkably well its original color. It was cured in the sun for two days; then spread out on the barn floor and allowed to dry thoroughly. When fed this hay was comparatively fresh and had not passed through the sweat of the mow.

### *2. Effect of direct sunlight*

Our previous experiments were conducted in the summer with most of the windows in the barn open. Immediately back of the cows were four open windows, allowing the direct sunshine to fall on the animals during part of the day. Hart and associates (5) have shown that the utilization of calcium and phosphorus by milking goats was increased by exposing the animals for twenty minutes daily to the emanations from a quartz mercury vapor lamp. As direct sunshine has been shown (6) to possess antirachitic properties similar to those exhibited by the rays from the quartz mercury vapor lamp, it is probable that this factor had a beneficial influence on the calcium and phosphorus utilization by our cows.

### *3. Favorable influence of beet pulp.*

The liberal use of beet pulp in our rations may well be listed among these factors. Gaessler and McCandlish (7) have noted positive calcium balances when beet pulp was a constituent of the ration, but they have attributed the favorable balances to the quality of the alfalfa hay fed at the same time. However, we do know that beet pulp has met with much favor as a feed for milking cows, and, owing to its comparatively high calcium content, it could be considered a mineral supplement. In our low protein rations approximately one-half of the calcium was supplied by beet pulp.

### *4. Effect of natural water*

In both of our experiments, natural, or tap water, was given the cows in preference to distilled water. This was done in order to avoid a change in the rations and to make the experimental conditions as nearly similar to the usual conditions as possible. In nearly all of the mineral metabolism experiments distilled water has been used. The kind of water used may or may not be a matter of great importance. As we had no knowledge of any definite experimental data regarding this factor, we thought it worthy of consideration.

### *5. History of cows*

Another very important factor was the history of the cows used in our work. These cows had never been allowed access to pasture or any other green feed. (They had received silage; but this is not generally considered a green feed). If it is true, as hypethecated by Forbes (8), that fresh, green grass (green roughage) with its "fresh grass vitamin" is necessary to the building up of large mineral reserves, then the status of our dry-feed cows regarding mineral reserves would be different from that of cows which had been allowed access to pasture or soilage. Presumably, most of the cows used in the other mineral metabolism experiments belonged to the latter class. The question naturally follows then, would this difference in the previous management of the cows lead to a difference in mineral balances.

## OBJECT

In this, our third mineral metabolism experiment, we have confined ourselves to a study of the last two factors discussed. The object of this work has been, therefore, twofold: First, to compare the balances of cows given distilled water with those of cows given natural or tap water, and, second, to compare the balances of cows that have been fed continually on winter rations with those of cows that have been allowed pasture in summer.

With these objects in view we planned this experiment in such a way as to either eliminate or equalize the effects of the other three factors, namely, the quality of the clover hay, direct sunshine, and beet pulp. This was accomplished by: First, feeding timothy hay as the roughage of the ration, and, second, eliminating direct sunlight by keeping the windows in the barn closed (this experiment was conducted in the winter). Third, equalizing the amounts of beet pulp in the rations.

## EXPERIMENTAL

### *Cows*

Four Holstein-Friesian cows were used in the work. Two of them, 146 and 154 were from the group of dry-feed cows and had been used in each of the former experiments. The other two, 163 and 203, were from the regular herd which had been pastured the preceding summer. In designating the two groups, we shall hereafter refer to the former as dry-feed cows and to the latter as regular herd cows.

Table 1 gives some of the more important facts concerning the cows. At the beginning of the experiment, cow 154 was in the fifth month of gestation; while 146 and 203 were in the second and first months, respectively. Cow 163 was bred during the interval between the periods of the experiment. Cow 203 freshened 343 days prior to the test; 154, 263 days; 146, 147 days; and 163, 118 days.

The average daily milk production during the experiment varied from 29 pounds for cow 154 to 32 pounds for 163; cows 146 and 203 taking intermediate positions with productions of 30 and 29.8 pounds respectively. It may be observed that these milk yields correspond very closely to the average daily yields for the entire lactations.

The average live weights for the six days at the beginning and end of the experiment are also given in table 1. Almost insignificant gains in weight are shown for cows 146 and 203. Cow 154 showed a significant gain of 51 pounds. No. 163 lost 26 pounds.

### *Experimental procedure*

The general methods used in this work were similar to those used in our former experiments (1).

The experiment consisted of two balance periods of eighteen days each, separated by an interval of twelve days. The only difference in the procedure in the two periods was in the nature of the water received by the cows, distilled water being given to one cow of each group, while the other cow of the group received natural water. This procedure was then reversed in the second period.

TABLE 1

*Data concerning cows used*

| | cow 146 | cow 154 | cow 163 | cow 203 |
|---|---|---|---|---|
| Breed | H. F. (Grade) | H. F. | H. F. | H. F. |
| Grouping | Dry-feed | Dry-feed | Regular herd | Regular herd |
| Age, years | 8.5 | 8.5 | 7.5 | 6 |
| Previous lactations | 5 | 5 | 4 | 1 |
| Freshened prior to test | 7/8/23 | 3/12/23 | 8/7/23 | 1/23/23 |
| Day of lactation at start of test | 147 | 263 | 118 | 343 |
| Live weight (average first six days of test) | 1,203 | 1,301 | 1,304 | 1,126 |
| Live weight (average last six days of test) | 1,206 | 1,352 | 1,278 | 1,130 |
| Date bred | 11/12/23 | 7/7/23 | 1/2/24 | 10/21/23 |
| Month of gestation (start of test) | 1 | 5 | 0 | 2 |
| Average daily milk production for test, pounds | 30 | 29 | 32 | 29.8 |
| Average daily milk production for lactation, pounds | 29.4 | 32 | 28 | 27.3 |
| Days in milk (lactation period) | 298 | 366 | 340 | 509 |

Each of these periods was divided into three 6-day sub-periods. Composite samples of milk, of urine, and of feces were made for each of the sub-periods. These composite samples were analyzed for the elements under consideration. Details for the sub-periods are not given here, because they showed no marked variation from the average daily figures here presented. Our purpose in basing the results on a number of samples rather than on one composite sample for each product was to reduce as much as possible the chances for error.

### *Sampling feeds and water*

The mineral and nitrogen analyses of the feeds and water are listed in table 2. Quantities of each of the grains and beet pulp sufficient for the entire experiment, were individually mixed, sacked and divided into two parts. One part was used in the first preliminary feeding and period I; The other was reserved for the second preliminary feeding and period II. Samples of each part were taken when the feeds were weighed for the individual rations. Separate analyses of the two sets of samples were made, but the results were so nearly identical that only one value is used.

TABLE 2

*Composition of feed—per cent (as weighed for rations)*

| FEED | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|
| Timothy hay, first period | 0.098 | 0.287 | 0.076 | 0.594 |
| Timothy hay, second period | 0.113 | 0.296 | 0.089 | 0.675 |
| Beet pulp | 0.081 | 0.696 | 0.290 | 1.410 |
| Corn | 0.222 | 0.008 | 0.110 | 1.490 |
| Bran | 1.616 | 0.127 | 0.621 | 2.360 |
| Oats | 0.399 | 0.081 | 0.147 | 2.030 |
| Molasses | 0.075 | 0.604 | 0.208 | 0.430 |
| Water, first period | | 0.0052 | 0.0019 | 0.00012 |
| Water, second period | | 0.0035 | 0.0012 | 0.00014 |

All the cane molasses used came from the same barrel. The analyses of the samples for each period were practically the same.

It was impossible to mix the timothy hay thoroughly because of its great bulk and the possbbility of shattering the heads. To compensate for this, we have taken a larger number of sub-samples. The difference in the composition of the hay used in the two periods is attributed largely to a greater contamination with clover in that fed during period II. The hay was chopped by running it through a silage cutter. This hay was of poor quality and had evidently been over-ripe when cut. It contained some coarse woody stems of weeds which the cows refused to eat. However, it was the best timothy hay obtainable at that time.

Water samples were taken daily. Aliquot samples representing the 6 sub-periods for each cow were made from these samples.

The distilled water was sampled and analyzed for its mineral content by the same methods used for tap water, the results in all cases being negligible. The natural water, though supposed to be entirely of deep well origin, contained a considerable amount of sediment following storms. Its mineral content was slightly lower in period II than in period I.

## *Rations*

The rations fed during the experiment are given in table 3. These rations were identical in composition and amount, with the one exception of a 5 per cent reduction for cow 203 during the second period. She consumed all of her feed during period I, but showed signs of being slightly overfed, as judged by her slow eating. This cow was the smallest of the experimental animals. No difficulty was experienced in getting the cows to consume completely the edible part of the rations. There was some refuse, which consisted largely of coarse weed stems found in the hay. This was carefully weighed and later analyzed. When computing the quantity of mineral matter contained in the refuse it was found that it did not amount to 0.1 gram a day, hence these figures are omitted in this discussion.

TABLE 3

*Mineral and nitrogen content of daily ration (grams)*

| FEED | AMOUNT* | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|---|
| | *pounds* | | | | | |
| Timothy hay | 10.5 | 4,762 | 4.7 | 13.7 | 3.6 | 28.3 |
| Beet pulp | 9.8 | 4,464 | 3.6 | 31.1 | 12.9 | 63.0 |
| Corn | 6.5 | 2,948 | 6.6 | 0.2 | 3.3 | 43.9 |
| Bran | 3.25 | 1,474 | 23.8 | 1.9 | 9.2 | 34.8 |
| Oats | 3.25 | 1,474 | 5.9 | 1.2 | 2.2 | 29.9 |
| Molasses | 1.3 | 590 | 0.4 | 3.5 | 1.2 | 2.5 |
| Total | | | 45.0 | 51.6 | 32.4 | 202.4 |

* Ration for no. 203 was 5 per cent less than this during period II.

The molasses was diluted with approximately an equal weight of water and sprinkled over the hay. This seemed to be very necessary in coaxing the cows to eat the hay. The dried sugar-beet pulp used in the rations was fed in the wet condition, having been soaked in 3 times its weight of water. The cows were extremely eager for the beet pulp. Yellow corn of good quality was fed in a finely ground condition. The oats were also ground. Water was given the cows "ad libitum." Nearly equal amounts of the two types of water were consumed in the course of the experiment. The slight difference of 0.9 per cent favored a larger consumption of natural water.

The mineral and nitrogen content of the daily rations is also given in table 3. The importance of beet pulp in the rations is here shown. This feed furnished approximately 60 per cent of the calcium, 40 per cent of the magnesium and 31 per cent of the nitrogen of the ration. Beet pulp contains very small amounts of phosphorus; 52 per cent of the phosphorus was supplied by the wheat bran.

Rations similar to those fed during the test had been fed to the two dry-feed cows (146 and 154) since their last freshening. The other two cows, (163 and 203), taken from the regular herd,[1] were fed these rations approximately 3 weeks prior to the beginning of the experiment. The cows were placed in the experimental stalls one week previous to the beginning of the experiment proper. Feeding of the specially weighed rations started at this time. The actual routine of the experiment had been in progress three days before the samples of milk and excreta were taken.

The data here presented were taken from December 5 to 23, 1923, for period I; and from January 4 to 22, 1924 for period II.

## DISCUSSION

In tables 4 to 7, inclusive, will be found the detailed figures relating to the mineral and nitrogen balances.

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[1] Ration: Grain mix (4 pints ground corn, 1 pint bran, and 1 pint oilmeal), 1 pound for every 3 pounds of milk produced; alfalfa hay, approximately 10 pounds per day.

TABLE 4

*Average daily balances of minerals and nitrogen (grams)*

Cow 146 (Dry-feed)

| | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|
| *Period I. Distilled water period (18 days)* | | | | | |
| Intake | | | | | |
| Feed | 15,712 | 45.0 | 51.6 | 32.4 | 202.4 |
| Water | 56,378 | | | | |
| Total intake | | 45.0 | 51.6 | 32.4 | 202.4 |
| Outgo | | | | | |
| Milk | 14,064 | 14.0 | 17.0 | 1.7 | 72.5 |
| Urine, cc | 6,771 | | 5.1 | 6.6 | 33.4 |
| Feces | 5,487 | 28.3 | 35.0 | 23.1 | 94.4 |
| Total outgo | | 42.3 | 57.1 | 31.4 | 200.3 |
| Balance | | +2.7 | −5.5 | +1.0 | +2.1 |
| *Period II. Natural Water Period (18 days)* | | | | | |
| Intake | | | | | |
| Feed | 15,712 | 45.7 | 52.0 | 33.0 | 206.3 |
| Water | 52,633 | | 1.9 | 0.7 | 0.1 |
| Total intake | | 45.7 | 53.9 | 33.7 | 206.4 |
| Outgo | | | | | |
| Milk | 13,149 | 13.0 | 15.5 | 1.4 | 67.2 |
| Urine. cc. | 5,355 | | 4.2 | 6.8 | 31.6 |
| Feces,. | 5,518 | 27.8 | 36.8 | 24.4 | 96.8 |
| Total outgo | | 40.8 | 56.5 | 32.6 | 195.6 |
| Balance | | +4.9 | −2.6 | +1.1 | +10.8 |
| *Average for both periods (36 days)* | | | | | |
| Intake | | | | | |
| Feed | 15,712 | 45.4 | 51.8 | 32.7 | 204.4 |
| Water | 54,506 | | 1.0 | 0.4 | |
| Total intake | | 45.4 | 52.8 | 33.1 | 204.4 |

TABLE 4—*Continued*

| | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|
| **Outgo** | | | | | |
| Milk | 13,607 | 13.5 | 16.3 | 1.6 | 69.9 |
| Urine. cc. | 6,063 | | 4.7 | 6.7 | 32.5 |
| Feces | 5,503 | 28.0 | 35.9 | 23.8 | 95.6 |
| Total outgo | | 41.5 | 56.9 | 32.1 | 198.0 |
| Balance | | +3.9 | −4.1 | +1.0 | +6.4 |

TABLE 5

*Average daily balances of minerals and nitrogen (grams)*

Cow 154 (Dry-feed)

| | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|
| *Period I. Natural Water Period (18 days)* | | | | | |
| **Intake** | | | | | |
| Feed | 15,712 | 45.0 | 51.6 | 32.4 | 202.4 |
| Water | 52,317 | | 2.6 | 1.0 | 0.1 |
| Total intake | | 45.0 | 54.2 | 33.4 | 202.5 |
| **Outgo** | | | | | |
| Milk | 13,957 | 14.3 | 15.4 | 1.8 | 70.8 |
| Urine, cc. | 8,549 | | 4.4 | 6.4 | 33.4 |
| Feces | 5,128 | 28.7 | 37.8 | 23.1 | 91.6 |
| Total outgo | | 43.0 | 57.6 | 31.3 | 195.8 |
| Balance | | +2.0 | −3.4 | +2.1 | +6.7 |
| *Period II. Distilled water period (18 days)* | | | | | |
| **Intake** | | | | | |
| Feed | 15,712 | 45.7 | 52.0 | 33.0 | 206.3 |
| Water | 50,650 | | | | |
| Total intake | | 45.7 | 52.0 | 33.0 | 206.3 |

TABLE 5—*Continued*

Outgo

| | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|
| Milk | 12,398 | 12.1 | 14.0 | 1.6 | 63.2 |
| Urine, cc | 6,798 | | 3.9 | 5.9 | 33.3 |
| Feces | 5,437 | 26.7 | 32.7 | 23.3 | 97.1 |
| Total outgo | | 38.8 | 50.6 | 30.8 | 193.6 |
| Balance | | +6.9 | +1.4 | +2.2 | +12.7 |

*Average for both periods (36 days)*

Intake

| | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|
| Feed | 15,712 | 45.4 | 51.8 | 32.7 | 204.4 |
| Water | 51,484 | | 1.3 | 0.5 | |
| Total intake | | 45.4 | 53.1 | 33.2 | 204.4 |

Outgo

| | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|
| Milk | 13,178 | 13.2 | 14.7 | 1.7 | 67.0 |
| Urine, cc | 7,674 | | 4.2 | 6.2 | 33.4 |
| Feces | 5,283 | 27.7 | 35.3 | 23.2 | 94.4 |
| Total outgo | | 40.9 | 54.2 | 31.1 | 194.8 |
| Balance | | +4.5 | −1.1 | +2.1 | +9.6 |

TABLE 6

*Average daily balances of minerals and nitrogen (grams)*

Cow 163 (regular herd)

*Period I. Distilled water period (18 days)*

Intake

| | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|
| Feed | 15,712 | 45.0 | 51.6 | 32.4 | 202.4 |
| Water | 53,450 | | | | |
| Total intake | | 45.0 | 51.6 | 32.4 | 202.4 |

TABLE 6—*Continued*

| | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|
| Outgo | | | | | |
| Milk | 14,816 | 15.1 | 18.1 | 1.8 | 76.1 |
| Urine, cc | 8,200 | | 7.0 | 8.1 | 39.9 |
| Feces | 4,819 | 30.7 | 36.2 | 21.5 | 92.4 |
| Total outgo | | 45.8 | 61.3 | 31.4 | 208.4 |
| Balance | | −0.8 | −9.7 | +1.0 | −6.0 |
| *Period II. Natural water period (18 days)* | | | | | |
| Intake | | | | | |
| Feed | 15,712 | 45.7 | 52.0 | 33.0 | 206.3 |
| Water | 54,933 | | 1.9 | 0.7 | 0.1 |
| Total intake | | 45.7 | 53.9 | 33.7 | 206.4 |
| Outgo | | | | | |
| Milk | 14,179 | 14.3 | 17.2 | 1.7 | 73.3 |
| Urine, cc | 6,448 | | 7.2 | 8.4 | 37.7 |
| Feces | 5,244 | 27.9 | 37.3 | 21.6 | 97.0 |
| Total outgo | | 42.2 | 61.7 | 31.7 | 208.0 |
| Balance | | +3.5 | −7.8 | +2.0 | −1.6 |
| *Average for both periods (36 days)* | | | | | |
| Intake | | | | | |
| Feed | 15,712 | 45.4 | 51.8 | 32.7 | 204.4 |
| Water | 54,192 | | 1.0 | 0.4 | |
| Total intake | | 45.4 | 52.8 | 33.1 | 204.4 |
| Outgo | | | | | |
| Milk | 14,498 | 14.7 | 17.7 | 1.8 | 74.7 |
| Urine, cc | 7,324 | | 7.1 | 8.3 | 38.8 |
| Feces | 5,032 | 29.3 | 36.8 | 21.6 | 94.7 |
| Total outgo | | 44.0 | 61.6 | 31.7 | 208.2 |
| Balance | | +1.4 | −8.8 | +1.4 | −3.8 |

TABLE 7

*Average daily balances of minerals and nitrogen (grams)*

Cow 203 (regular herd)

| | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|
| *Period I. Natural water period (18 days)* | | | | | |
| Intake | | | | | |
| Feed | 15,712 | 45.0 | 51.6 | 32.4 | 202.4 |
| Water | 55,495 | | 2.9 | 1.1 | 0.1 |
| Total intake | | 45.0 | 54.5 | 33.5 | 202.5 |
| Outgo | | | | | |
| Milk | 13,464 | 13.5 | 15.5 | 1.9 | 73.5 |
| Urine, cc | 4,088 | | 5.8 | 8.6 | 31.7 |
| Feces | 5,601 | 30.4 | 41.3 | 21.8 | 100.9 |
| Total outgo | | 43.9 | 62.6 | 32.3 | 206.1 |
| Balance | | +1.1 | −8.1 | +1.2 | −3.6 |
| *Period II. Distilled water period (18 days)* | | | | | |
| Intake | | | | | |
| Feed | 14,929 | 43.4 | 49.4 | 31.4 | 196.0 |
| Water | 52,833 | | | | |
| Total intake | | 43.4 | 49.4 | 31.4 | 196.0 |
| Outgo | | | | | |
| Milk | 13,557 | 13.5 | 16.0 | 2.0 | 71.7 |
| Urine, cc | 3,960 | | 6.2 | 7.7 | 29.1 |
| Feces | 5,603 | 29.1 | 36.1 | 22.2 | 95.0 |
| Total outgo | | 42.6 | 58.3 | 31.9 | 195.8 |
| Balance | | +0.8 | −8.9 | −0.5 | +0.2 |
| *Average for both periods (36 days)* | | | | | |
| Intake | | | | | |
| Feed | 15,320 | 44.2 | 50.5 | 31.9 | 199.2 |
| Water | 54,164 | | 1.5 | 0.6 | |
| Total intake | | 44.2 | 52.0 | 32.5 | 199.2 |

TABLE 7—*Continued*

| | AMOUNT | PHOSPHORUS | CALCIUM | MAGNESIUM | NITROGEN |
|---|---|---|---|---|---|
| Outgo | | | | | |
| Milk | 13,510 | 13.5 | 15.8 | 2.0 | 72.6 |
| Urine, cc | 4,024 | | 6.0 | 8.1 | 30.1 |
| Feces | 5,602 | 29.8 | 38.7 | 22.0 | 98.0 |
| Total outgo | | 43.3 | 60.5 | 32.1 | 200.7 |
| Balance | | +0.9 | −8.5 | +0.4 | −1.5 |

## *Phosphorus balances*

Retention of this element was shown in 7 cases out of 8; the one negative balance, that of cow 163, period I, amounted to only 0.8 gram. The largest storage of phosphorus occurred in the balance for cow 154 during the second period: this daily retention of 6.9 grams was accompanied by a storage of all the elements studied, together with a gain in weight and decline in milk production.

The type of water given the cows apparently had little influence on the phosphorus balances. Greater quantities of this element were retained by 3 of the 4 cows during period II than during period I. In the case of the fourth cow the decrease in positive balance, amounting to 0.3 gram, does not seem significant in view of the fact that her ration, hence her phosphorus intake, had been reduced. As two of the cows received natural water during the second period, and the other two distilled water (both sets having been reversed in this respect from the first period), it is evident that the phosphorus balances show no relationship to the type of water used.

We believe that the increased positive balances for the 3 cows during period II can be explained largely by the decrease in milk production during this period. The increase in milk production by the fourth cow was accompanied by a decrease in storage. It must also be remembered that the ration of this cow (203, period II) had been slightly reduced. Data bearing on the relationship between the changes in milk production and the phosphorus balances are given in table 8.

A comparison of the phosphorus balances for the two groups shows that, for the entire 36 days of the experiment, the dry-feed group had larger positive balances than the regular herd group. The average daily retentions by the dry-feed cows, 146 and 154, were 3.9 and 4.5 grams, respectively; while those of the regular herd cows, 163 and 203, were 1.4 and 0.9 gram, respectively. It is true that the milk production for the latter group slightly exceeded that for the former; but this difference was not sufficient to account for the variation in the balances.

TABLE 8

| COW | DECLINE IN MILK YIELD | INCREASE IN PHOSPHORUS BALANCE | RATIO |
|---|---|---|---|
| | *grams* | *grams* | |
| 146 | 915 | 2.2 | 1:0.0024 |
| 154 | 1,559 | 4.9 | 1:0.0031 |
| 163 | 637 | 4.3 | 1:0.0067 |
| 203 | 93 (Increase) | 0.3 (Decrease) | 1:0.0032 |

## CALCIUM BALANCES

There was only one storage of calcium and that was for cow 154 during the second period, the average daily retention in this case being 1.4 grams. The losses varied from 2.5 grams for cow 146, period II, to 9.7 grams for 163, period I.

The calcium balances for cows 146, 154, and 163 were more favorable during the second half of the work than during the first half. Like the phosphorus balances, it seems that the general difference in the calcium balances can be very largely explained by the decline in milk production. As a matter of interest, the relationship between the change in calcium balances and the decline in milk production is presented in table 9. This table shows that for every gram decline in milk there was an increase in the calcium balance amounting to approximately 3 mgm.

When considering the nature of the water used in mineral metabolism experiments as a possible factor affecting the assimilation of minerals, it was believed that the effects of the natural water would be more evident in the calcium balances than in the other mineral balances, because calcium was the element found in the largest quantities in the water. As previously indicated, the data for period II show more favorable calcium balances than those for period I, regardless of the nature of the water used. Therefore, it would seem fair to assume that the natural water had no marked effect on the utilization of this element.

For the entire thirty-six days the average daily balances were more favorable for the dry-feed group than for the regular herd group. Though the regular herd cows produced slightly more milk, the difference in milk production was not sufficient to account for the difference in calcium balances. These results compare as follows: the dry-feed cows, 146 and 154 lost approximately 4 and 1 gram per day, respectively; while the regular herd cows, 163 and 203, each lost approximately 8.5 grams daily.

TABLE 9

| COW | DECLINE IN MILK YIELD | INCREASE IN CALCIUM BALANCE | RATIO |
|---|---|---|---|
| | *grams* | *grams* | |
| 146 | 915 | 2.9 | 1:0.0031 |
| 154 | 1,559 | 4.8 | 1:0.0031 |
| 163 | 637 | 1.9 | 1:0.0030 |
| 203 | 93 (Increase) | 0.8 (Decrease) | 1:0.0086 |

### *Magnesium balances*

Of the eight balances for this element, only one was negative, and this only to the extent of 0.5 gram daily. This loss was shown by cow 203, during the second period. Only a slight variation was shown in the 7 positive balances: the smallest storage was that of cow 146, period I, amounting to 1 gram; and the largest, that of cow 154, period II, amounting to 2.2 grams.

The type of water used had no effect on the magnesium balances. It seemingly had an influence on the outgo of this element in the urine, the average daily quantity for the individual cows being slightly increased when natural water was used.

Approximately 1 gram of magnesium was supplied daily by the water.

A comparison of the magnesium balances of the two groups for the thirty-six days does not show that any distinctive difference existed between them. It is, interesting to note the similarity in magnesium outgo in the urine of the two cows in each group. Greater amounts of this element were found in the urine of the regular herd group.

### *Nitrogen balances*

The nitrogen balances were positive for the two dry-feed cows in both periods; and negative, except in one instance, for the regular herd cows. The one storage of nitrogen, in the latter group, was less than 0.5 gram per day. Variations from a 12.7 grams retention for cow 154, period II, to a 6 grams loss for cow 163, period I are shown.

The average daily data for the entire time covered by the experiment show positive balances of 6.4 and 9.6 grams for the dry-feed cows, 146 and 154; and negative balances of 3.8 and 1.5 gram for the regular herd cows, 163 and 203.

The type of water used in the ration had no apparent effect on the nitrogen balances.

This work shows that the calcium, phosphorus, and nitrogen balances are influenced to some extent at least by the previous feeding of the cows. In our opinion the difference in the previous feeding of the two groups had affected the amounts of the available elements stored in their bodies. It seems logical to assume that the dry-feed cows, which had long received these rations known to be conducive to mineral and nitrogen losses, would have smaller reserves than the regular herd cows, which had received the same rations for only three weeks previous to the experiment. (The latter group had been changed from a ration containing alfalfa hay and a more liberal protein supply.) If this assumption is correct, then the extent of the positive and negative balances was influenced to some degree by the amounts of available elements in reserve, as shown in the data here reported. In this light, we would interpret some of the large negative balances that have been observed as indicating losses in extensive reserves rather than as proving that a fairly liberal milk production (30 pounds per day) is impossible without large losses of minerals and nitrogen, even under winter conditions.

That the dry-feed cows averaged almost 30 pounds of milk per day is shown by the records for their fourth, fifth, and sixth lactations, or those periods during which our experiments were conducted (table 10; see table 1, also for the sixth lactation). The rations received by these cows during the sixth lactation were the same as those used in this experiment. In the fourth and fifth lactations, rations containing some clover hay were given. The details of this feeding are given in our former paper (1).

TABLE 10

*Summary of milk and fat production for dry-feed cows, (fourth, fifth, and sixth periods)*

| COW | MILK PRODUCTION | FAT PRODUCTION | DAYS IN MILK | DAYS DRY | AVERAGE DAILY MILK PRODUCTION | AVERAGE WEIGHT OF CALVES |
|---|---|---|---|---|---|---|
| | *pounds* | *pounds* | | | *pounds* | *pounds* |
| 146* | 30,067 | 1,162 | 1,028 | 218 | 29.1 | 107 |
| 154 | 31,220 | 1,040 | 1,073 | 174 | 29.1 | 119 |

* Aborted seven months, calf weighing 106 pounds, in 1924.

## *Summary of discussion*

Eight balances of phosphorus, calcium, magnesium, and nitrogen are reported. Four of these balances were determined on cows that had always been restricted to dry feeds; and four on cows taken from the regular herd, which had been pastured the preceding summer. A comparison was made of the balances of cows given distilled water with those of cows given natural or deep well water. The double reversal method was used for making this comparison. All rations were otherwise identical with the exception of a 5 per cent reduction in that of one cow during the latter part of the work. The level of daily milk production was approximately 30 pounds.

The calcium balances formed the chief difference between the dry-feed and regular herd groups. Taking the entire thirty-six days as the unit, all these balances were negative; those of the regular herd cows, amounting to approximately 8.5 grams daily, were more than double the losses sustained by the dry-feed cows.

The regular herd cows lost nitrogen at the rate of 1.5 and 3.8 grams daily; while the dry-feed cows stored 6.4 and 9.6 grams.

The phosphorus balances showed that the regular herd cows retained only 0.9 and 1.4 grams daily, while the dry-feed cows retained 3.9 and 4.5 grams daily.

No difference was found between the two groups in respect to the magnesium balances.

The type of water used in the rations had no apparent effect on the balances of any of the elements studied. The daily calcium intake from the natural water varied from 2 to 3 grams, while the magnesium intake from this source approximated 1 gram daily.

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# EFFECT OF BONE MEAL ON GROWTH OF DAIRY HEIFERS*

W. D. SALMON AND W. H. EATON

*Department of Animal Industry, Alabama Polytechnic Institute, Auburn, Alabama*

The literature contains considerable data on the effect of calcium and phosphorus compounds on milking cows with reference to the milk production and the maintenance of equilibrium of these elements. The data on the effect of such compounds on the growth of dairy animals are very meager.

Hart (2) et al. report that the addition of bone meal and cod liver oil to rations made up of products of the wheat plant gave a marked improvement in the growth and reproduction of dairy cows.

Eckles (1) reports that a Jersey heifer receiving a ration low in mineral made a growth both in height and weight equal to a similar heifer receiving a ration that contained approximately three times as much calcium and phosphorus. The heifer on the low mineral ration suffered a break down in condition at the age of 18 months and it was necessary to change the ration.

Sir Arnold Theiler (3) has found that the feeding of bone meal has a very favorable effect on the gains of cattle that are grazed on native grasses in South Africa where the soil contains very little calcium and phosphorus and where lack of sufficient moisture makes conditions very unfavorable for the assimilation of these constituents by plants.

The problem of whether our common farm rations can be improved by the addition of various mineral supplements is an important one. An experiment to test the effect of adding bone meal to a ration which is often fed dairy heifers was begun at this station in December, 1922.

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* Received for publication March 6, 1925.

## EXPERIMENTAL

### *Plan*

Two groups of 4 heifers each were used. The groups were fairly uniform with respect to individuality of the animals. Both groups received a grain mixture of 300 pounds of shelled corn and 200 pounds of oats. The hay given consisted chiefly of Johnson Grass and timothy. Both groups had access to block salt and one group received 2 ounces of steamed bone meal mixed in grain each day. Tables 1 and 2 show feed records by periods of twenty-eight days.

TABLE 1

*Feed consumed by periods (December 10, 1922, to April 26, 1924, inclusive)*

Lot 1—2 ounces bone meal daily

| PERIODS 28 DAYS EACH | HEIFER 45 | | HEIFER 47 | | HEIFER 49 | | HEIFER 43 | |
|---|---|---|---|---|---|---|---|---|
| | Grain | Hay | Grain | Hay | Grain | Hay | Grain | Hay |
| | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* |
| 1 | 112 | 21 | 112 | 21 | 112 | 21 | 112 | 21 |
| 2 | 145.5 | 48 | 145 | 48 | 145 | 48 | 145 | 48 |
| 3 | 168 | 56 | 168 | 56 | 168 | 56 | 168 | 56 |
| 4 | 168 | 56 | 168 | 56 | 168 | 56 | 155 | 56 |
| 5 | 168 | 56 | 168 | 56 | 168 | 56 | 168 | 56 |
| 6 | 115 | 56 | 115 | 56 | 114 | 56 | 111.5 | 56 |
| 7 | 112 | 56 | 112 | 56 | 112 | 56 | 111 | 56 |
| 8 | 84 | 54 | 81.5 | 54 | 81.5 | 54 | 84 | 54 |
| 9 | 82.5 | 56 | 82.5 | 56 | 80.5 | 56 | 81.5 | 56 |
| 10 | 84. | 56 | 83 | 56 | 84 | 56 | 84 | 56 |
| 11 | 84 | 56 | 84 | 56 | 84 | 56 | 84 | 56 |
| 12 | 103 | 56 | 101 | 56 | 104 | 56 | 104 | 56 |
| 13 | 96 | 48 | 97 | 54 | 98 | 56 | 102 | 56 |
| 14 | Calved and ration changed | | 150 | 56 | 150 | 56 | 150 | 56 |
| 15 | | | 168 | 56 | 168 | 56 | 168 | 56 |
| 16 | | | 168 | 56 | 168 | 56 | 168 | 56 |
| 17 | | | 168 | 56 | 168 | 56 | 168 | 56 |
| 18 | | | 140 | 56 | 140 | 56 | 140 | 56 |
| Total... | 1,522 | 675 | 2,311 | 961 | 2,313 | 963 | 2,304.0 | 963 |

During the grazing season the heifers were on Bermuda, carpet grass, and dallis grass pasture. This pasture was on acid land of low lime content. Weights and measurements were taken every twenty-eight days; although only a part of the data are presented.

## *Results*

Curves of growth of the heifers in weight and height are shown in figures 1 and 2. The feeding of 2 ounces of bone meal per day had very little effect on the rate of growth. Apparently the Jerseys that received the bone meal increased slightly faster in weight than those that did not receive bone meal. The two Holsteins showed exactly the reverse. There was a very close agreement in the height curves of the two groups.

TABLE 2

*Feed consumed by periods (December 10, 1922, to April 26, 1924, inclusive)*

Lot 2—no mineral

| PERIODS 28 DAYS EACH | HEIFER 46 | | HEIFER 48 | | HEIFER 41 | | HEIFER 42 | |
|---|---|---|---|---|---|---|---|---|
| | Grain | Hay | Grain | Hay | Grain | Hay | Grain | Hay |
| | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* |
| 1 | 112 | 21 | 112 | 21 | 112 | 21 | 112 | 21 |
| 2 | 140.5 | 48 | 145 | 48 | 137.3 | 48 | 138.5 | 48 |
| 3 | 168 | 56 | 168 | 56 | 125.5 | 56 | 168 | 56 |
| 4 | 168 | 56 | 168 | 56 | 155 | 56 | 168 | 56 |
| 5 | 168 | 56 | 165 | 56 | 164.8 | 52 | 168 | 56 |
| 6 | 115 | 56 | 115 | 56 | 110 | 56 | 112 | 56 |
| 7 | 112 | 56 | 112 | 56 | 106 | 56 | 112 | 56 |
| 8 | 84 | 54 | 72 | 54 | 81.5 | 54 | 81.5 | 54 |
| 9 | 82.5 | 56 | 82.5 | 56 | 82.5 | 56 | 82.5 | 56 |
| 10 | 84 | 56 | 84 | 56 | 84 | 56 | 84 | 56 |
| 11 | 84 | 56 | 84 | 56 | 82.5 | 56 | 84 | 56 |
| 12 | 104 | 56 | 104 | 56 | 93.5 | 56 | 104 | 56 |
| 13 | 104 | 56 | 93 | 54 | 98 | 54 | 98 | 56 |
| 14 | Calved and ration changed | | 150 | 56 | 150 | 56 | 150 | 56 |
| 15 | | | 168 | 56 | 168 | 56 | 168 | 56 |
| 16 | | | 168 | 56 | 168 | 56 | 168 | 56 |
| 17 | | | 168 | 56 | 168 | 56 | 168 | 56 |
| 18 | | | 140 | 56 | 140 | 56 | 140 | 56 |
| Total... | 1,526 | 683 | 2,298.5 | 961 | 2,226.6 | 957 | 2,306.5 | 963 |

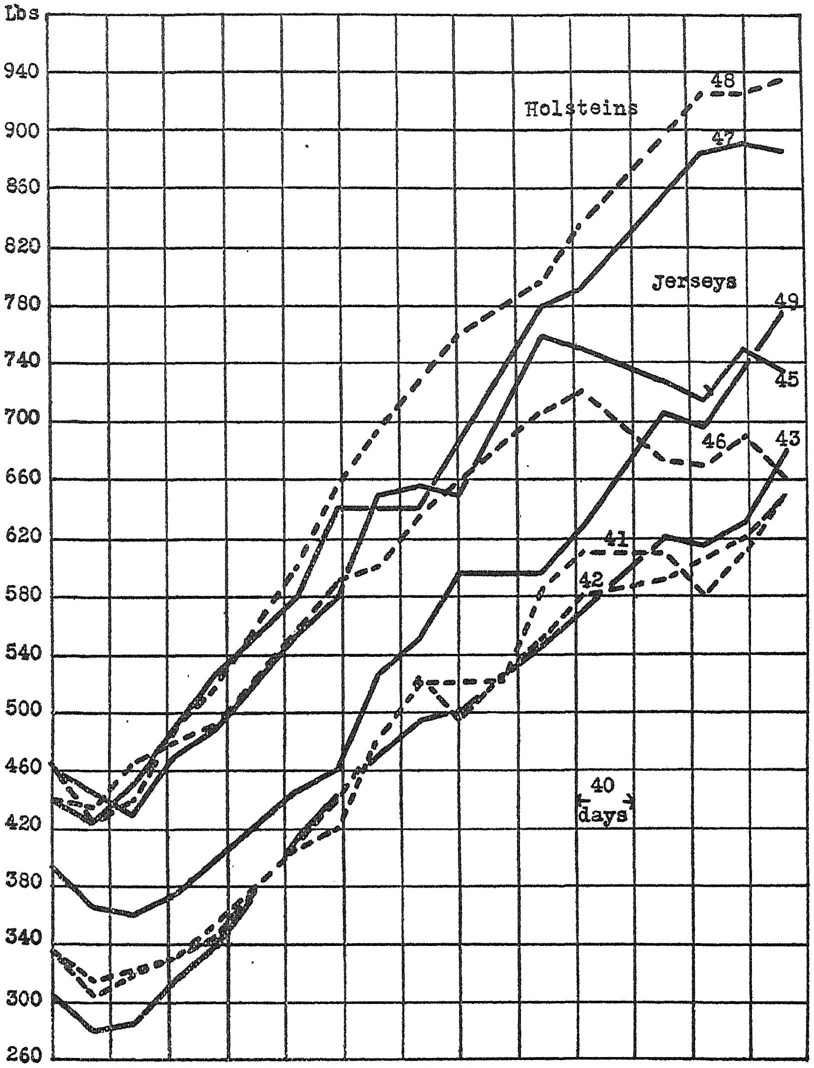

FIG. 1. GROWTH CURVES OF HEIFERS—WEIGHT
Solid lines indicate heifers that received 2 ounces bone meal daily

## DISCUSSION

The data presented can in no way be considered conclusive due to the small number of individuals used. It is shown however that the addition of 2 ounces of steamed bone meal per animal per day did not have a decided effect on the growth in weight or height of 3 Jerseys and 1 Holstein. The character of growth was very similar to that of comparable heifers that did not receive any mineral supplement except common salt.

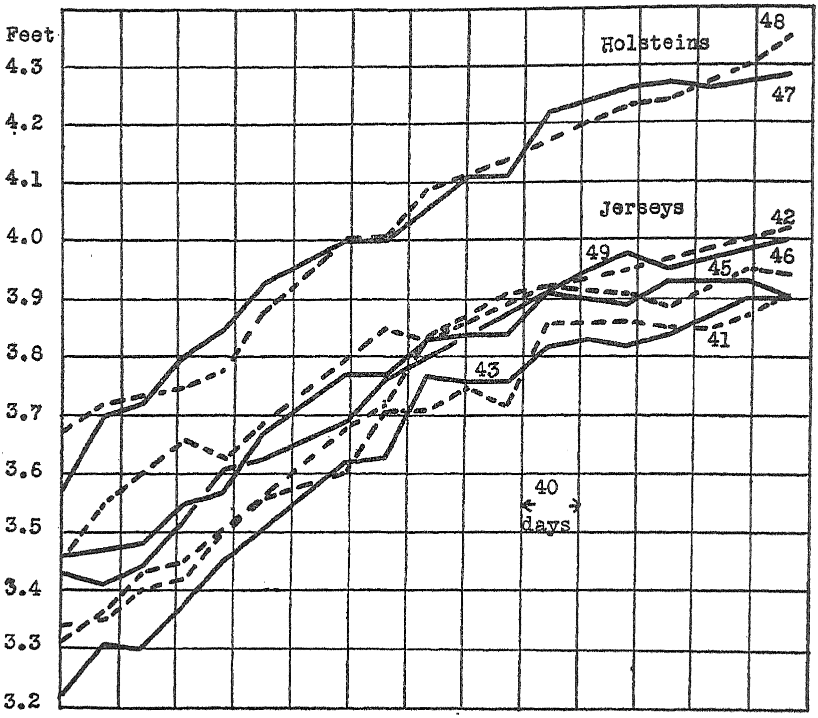

FIG. 2. GROWTH CURVES OF HEIFERS—HEIGHT
Solid lines indicate heifers that received 2 ounces bone meal daily

The ration used was rather low in calcium and the pasture was on land that has a low lime content. In view of these facts we expected the feeding of bone meal to be advantageous.

As the heifers calved they were placed in the milking[1] herd and the allowance of bone meal increased to 4 ounces daily. Birth weights and measurements of the calves were taken and the heifer calves will be continued on the same plan of mineral feeding as the dams. It is possible that the effect of mineral feeding will be reflected in the milk production or that it will show up in later generations.

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[1] Our milking herd consists of 3 groups: One group receives no mineral supplement except salt; one group receives salt and 4 ounces of bone meal per cow daily; and the other group receives salt and 4 ounces of $CaCO_3$ per cow daily.

# CARBONATION OF BUTTER*

M. J. PRUCHA, J. M. BRANNON AND H. A. RUEHE

*Department of Dairy Husbandry, University of Illinois, Urbana, Illinois*

This study on the carbonation of butter is a part of a larger problem under investigation, namely, that of "The deterioration of dairy products and the means of prevention." As originally planned, the study included the use of other gases, particularly O, N and H. After some preliminary experiments all the other gases except carbon dioxide were given up because of the unfavorable results.

Carbon dioxide is used extensively and with success in the preparation of certain beverages (1, 2, 3, 4, 5, 6). Attempts have also been made to extend its use to the dairy products with the expectation that certain benefits would be derived therefrom. Van Slyke and Bosworth (7), published their study on the carbonation of milk in 1907. They showed that the charging of milk with the gas and keeping it under pressure delayed the souring of the milk. One of the authors, M. J. Prucha, who was then connected with the same institution, became interested in the subject after examining bacteriologically several samples of the carbonated milk. Since that time, the use of carbon dioxide for dairy products and other foods has been patented (8, 9). The dairy industry suffers heavy losses from the spoiling and the deterioration of the dairy products. Hence, any scheme, the aim of which is to improve these products and to prevent their spoilage, should receive careful consideration. A study on this subject was organized and has been carried on for more than three years. One report of this work, dealing with the carbonation of ice cream, has already been published (10). This paper presents the results from the experiments on the carbonation of butter.

Three different series of experiments were carried out. In the first series the carbon dioxide was applied to the sweet cream which was intended for butter. In the second series of experi-

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* Received for publication May 4, 1925.

ments the carbon dioxide was applied at the time of churning, the butter being churned in the atmosphere of the gas. In the third series of experiments the butter was stored in carbon dioxide.

The main object of these experiments was to determine the effects of carbonation of cream and butter on the germ life, on the development of flavors, and on the keeping quality of these products.

The bacteriological work included both the counting of the bacteria, and the examination of the petri plates as to the kind of microbial flora that survived the carbonation. In some of the experiments yeast and mold counts were also made. Nutrient agar containing two per cent lactose was used for the bacteria, and acidified malted agar was used for the yeasts and the molds.

## CARBONATION OF SWEET CREAM

The experiments with the cream were conducted in a laboratory. Usually about 4 gallons of sweet cream were divided into four lots, two of which were carbonated and the other two were not. The cream was kept at two different temperatures, namely, at 40° and 70°F.

The carbonation was done as follows: A rubber hose was attached to the gas tank and to the loose end of the hose was attached a glass tube 2 feet long. The loose end of the glass tube was inserted into the cream as far as the bottom. The gas was then allowed to flow into the cream for three minutes at 5 pounds pressure. The cream was being stirred during the flow of the gas. The carbonation was repeated each day during the experiment.

At varying intervals of time samples of the cream were taken for the bacteriological examination and also for the acidity test. Two samples were always taken for the acidity test, one of which was heated to expel the carbon dioxide before applying the test.

The experiment was repeated at least ten times in addition to several preliminary trials. The results in all cases were so similar that only the results from one of the experiments are presented (table 1).

There are two points to be noticed in the data in table 1 as regards the influence of carbon dioxide on the bacteria in the cream. In the first place, the carbonation caused in most cases, but not always, a slight temporary reduction in the number of bacteria following the first application of the gas. The second point to be noticed is that the carbonation did not prevent the multiplication of the bacteria. Other experiments showed that no amount of carbonation, unless under high pressure, will stop the bacteria from multiplying. After the temporary slight reduction the bacteria increased at about the same rate as they did in the uncarbonated cream.

TABLE 1

*Effect of carbon dioxide on bacteria and on acidity in cream*

| AGE OF CREAM | CARBONATED | | | UNCARBONATED | | |
|---|---|---|---|---|---|---|
| | Number of bacteria per cubic centimeter | Acidity | | Number of bacteria per cubic centimeter | Acidity | |
| | | Before heating | After heating | | Before heating | After heating |
| Cream kept at room temperature | | | | | | |
| Fresh | 9,000,000 | 0.38 | 0.12 | 9,000.000 | 0.12 | 0.12 |
| 2 hours | 6,000,000 | | | 16,000,000 | | |
| 4 hours | 24,000,000 | | | 28,000,000 | | |
| 6 hours | 21,000,000 | | | 19,000,000 | | |
| 8 hours | 46,000,000 | | | 41,000,000 | | |
| 1 day | | 0.56 | 0.49 | | 0.49 | 0.44 |
| 2 days | 346,000,000 | 0.68 | 0.53 | 221,000,000 | 0.66 | 0.56 |
| 4 days | 245,000,000 | 0.81 | 0.51 | 307,000,000 | 0.60 | 0.49 |
| | | 0.65 | 0.45 | | 0.55 | 0.49 |
| Same cream as above but kept at 40°F. | | | | | | |
| Fresh | 9,000,000 | 0.28 | 0.12 | 9,000,000 | 0.12 | 0.12 |
| 3 hours | 11,000,000 | | | 17,000,000 | | |
| 6 hours | 12,000,000 | | | 18,000,000 | | |
| 8 hours | 8,000,000 | | | 23,000,000 | | |
| 1 day | | 0.28 | 0.14 | | 0.16 | 0.15 |
| 2 days | 35,000,000 | 0.50 | 0.16 | 55,000,000 | 0.25 | 0.24 |
| 4 days | 23,000,000 | 0.55 | 0.31 | 114,000,000 | 0.45 | 0.45 |
| 6 days | 103,000,000 | 0.7 | 0.41 | 312,000,000 | 0.50 | 0.43 |
| 7 days | | 0.7 | 0.42 | | 0.53 | 0.48 |

From the examination of the petri plates prepared from the cream, it was very evident that the carbonated cream developed somewhat different bacterial flora than the uncarbonated. Apparently the carbon dioxide checks certain types of bacteria and not other types. This probably explains the slight reduction due to carbonation.

The acidity test likewise showed that there was at first a slight retardation in the development of the acid in the carbonated cream; but, it also showed that this delay in the development of acidity was of short duration, and that both the carbonated and the uncarbonated cream soured, and that the degree of acidity in the end was about the same. It should also be mentioned that the uncarbonated cream became partially carbonated as it aged, due to the production of carbon dioxide through the activity of the germ life in the cream.

The effect of the carbonation on the keeping quality of the cream was slight and depended on the temperature at which the cream was held and probably on the amount and on the kind of initial bacterial contamination. At room temperature the carbonated cream soured in about the same length of time as the uncarbonated, a matter of two or three hours difference. At 40°F. the carbonated cream kept about two or three days longer.

From the commercial standpoint the carbonation of sweet cream for the purpose of improving the butter would not be practical. The diffculty of applying the carbon dioxide at the source of cream production, the expense involved, and the slight and doubtful benefits derived, would not make it economically profitable.

## CARBONATION OF BUTTER WHILE CHURNING

The experiments on the carbonation of butter while churning were conducted both in the laboratory and also in a commercial creamery. The carbon dioxide was applied in the following manner. In the laboratory experiments, a rubber hose was attached to the gas tank and the loose end of the hose was inserted into the cream as far as the bottom of the churn. The gas was then allowed to flow into the cream during the entire churning operation. In the creamery experiments, a four foot metal pipe was connected to the gas tank by means of a rubber hose. The loose end of the pipe was inserted in the cream as far as the bottom of the churn and the gas was then blown in while the cream was being stirred until the cream was saturated and the gas escaped from the churn through the top opening. The churn was then closed and the churning operation was carried out as usual.

Approximately thirty experiments were done in the laboratory. In addition to the bacteriological study, the butter was stored at different temperatures and its keeping quality and the development of flavors were observed.

These laboratory experiments revealed no pronounced effect of the carbonation on the bacteria in the butter. The lactic acid bacteria, which constitute a large percentage of the bacteria in properly ripened cream, did not appear to be affected by the carbon dioxide.

The carbonated butter had a sourish taste immediately after the churning. This taste gradually disappeared in storage. In regard to the keeping quality of the butter, there was no pronounced difference between the carbonated and the uncarbonated butter except that the former tended to develop a slightly different off flavor. In these laboratory experiments it was impossible to find any benefits due to the carbonation that would be valuable enough to pay for the cost of the treatment.

The experiments in the creamery were done on a large scale and in the following manner. On three different days about 3000 gallons of cream were put in a holding tank after the cream was neutralized, pasteurized, and inoculated with a starter. The ripened cream was then drawn from this tank into the churns. The first three churnings, amounting to 2400 pounds of butter, were not treated. In the next three churnings the carbon dioxide was applied to the cream in the churns. The remaining cream was then carbonated in the holding tank. From each churning a sixty pound tub of butter was placed in cold storage at 40°F. from which to study the changes in the quality of the butter. The butter was scored by an expert judge at varying intervals of time. In one experiment the butter was held in the cold storage for 135 days and in the other two experiments only

TABLE 2

*Effect of carbonation on the keeping quality of butter*

| NUMBER OF CHURN | TREATMENT OF THE BUTTER | AGE OF THE BUTTER | | | | |
|---|---|---|---|---|---|---|
| | | 6 days | 20 days | 40 days | 90 days | 135 days |
| | | Butter score | | | | |
| | First experiment | | | | | |
| 1 | Not salted, not carbonated | 89 | 88 | 88 | 87.5 | 88.5 |
| 2 | Salted, not carbonated | 89.5 | 88.5 | 88.5 | 88.5 | 88.5 |
| 3 | Salted, not carbonated | 88.5 | 89 | 88 | 86.5 | 87 |
| 4 | Not salted, carbonated | 89.5 | 89 | 89 | 88 | 88 |
| 5 | Not salted, carbonated | 89.5 | 89 | 89 | 89 | 87.5 |
| 6 | Salted, carbonated | 88.5 | 89 | 88.5 | 87 | 87 |
| 7 | Salted, carbonated | 89 | 89.5 | 88 | 88 | 87.5 |
| 8 | Salted, carbonated | 89 | 90 | 88.5 | 88 | 88 |
| 9 | Salted, carbonated | 89.5 | 89.5 | 88.5 | 87 | 87.5 |
| 10 | Salted, carbonated | 89 | 87.5 | 86.5 | 86 | 87 |
| | Second experiment | | | | | |
| 1 | Not salted, not carbonated | 88.5 | 89.5 | 88 | | |
| 2 | Not salted, not carbonated | 90 | 88.5 | 87.5 | | |
| 3 | Not salted, not carbonated | 89 | 88 | 88 | | |
| 4 | Salted, carbonated | 88.5 | 88 | 88.5 | | |
| 5 | Salted, carbonated | 89.5 | 87 | 88.5 | | |
| 6 | Not salted, carbonated | 89.5 | 88 | 87.5 | | |
| 7 | Salted, carbonated | 90 | 88 | 88 | | |
| 8 | Salted, carbonated | 90 | 87 | 89 | | |
| 9 | Salted, carbonated | 90 | 88 | 88.5 | | |
| 10 | Salted, carbonated | 90.5 | 88 | 89 | | |
| 11 | Salted, carbonated | 87 | 86.5 | 84 | | |
| 12 | Salted, carbonated | 87 | 87 | 85 | | |
| | Third experiment | | | | | |
| 1 | Salted, not carbonated | 87 | 87 | 88 | | |
| 2 | Salted, not carbonated | 88 | 87 | 87.5 | | |
| 3 | Salted, not carbonated | 89 | 87.5 | 88.5 | | |
| 4 | Salted, carbonated | 89.5 | 88.5 | 87.5 | | |
| 5 | Salted, carbonated | 89.5 | 88.5 | 88.5 | | |
| 6 | Salted, carbonated | 90 | 88 | 88.5 | | |
| 7 | Salted, carbonated | 90 | 88.5 | 89 | | |
| 8 | Salted, carbonated | 88 | 88 | 88.5 | | |

*Note:* In churnings numbered 7 to 12 the cream was carbonated in the holding vat.

forty days. The results of the scoring of the butter are presented in table 2.

The numerical scores given to the butter by the judge as shown in table 2, do not reveal any measurable benefits nor any harm due to the carbonation. The carbonated butter would not have commanded a better price on the market than the uncarbonated. In some cases the carbonated butter was pronounced sourish. One lot of butter was made from cream which was slightly over neutralized and in this case the carbonation tended to cover this defect. The butter that was salted was free from molds while the unsalted butter became slightly moldy whether it was carbonated or uncarbonated.

TABLE 3

*Number of bacteria, molds and yeasts in carbonated and uncarbonated cream and butter*

| CHURNING | NUMBER OF BACTERIA | | NUMBER OF YEASTS AND MOLDS | |
|---|---|---|---|---|
| | Per cubic centimeter cream | Per gram butter | Per cubic centimeter cream | Per gram butter |
| Not carbonated | | | | |
| 1 | 17,000,000 | 4,000,000 | 310 | 300 |
| 2 | 31,000,000 | 3,000,000 | 420 | 390 |
| 3 | 36,000,000 | 600,000 | 490 | 610 |
| Carbonated in churn | | | | |
| 4 | 33,000,000 | 2,000,000 | 310 | 1,400 |
| 5 | 32,000,000 | 4,000,000 | 480 | 240 |
| 6 | 32,000,000 | 2,000,000 | 390 | 240 |
| Cream carbonated in holding tank | | | | |
| 7 | 12,000,000 | 2,000,000 | 410 | 300 |
| 8 | 27,000,000 | | 290 | 400 |
| 9 | 25,000,000 | 3,000,000 | 230 | 800 |
| 10 | 14,000,000 | 4,000,000 | 5,600 | 800 |

Bacteriological counts were made from the cream immediately before churning and also from the butter when it was taken from the churn. The results from one day's run are given in table 3.

The bacteriological results show no significant effect of the carbonation. If any effect was produced it was not large enough to be detected by the bacteriological methods.

### STORING OF BUTTER IN CARBON DIOXIDE

In this series of experiments freshly made butter was placed in an atmosphere of carbon dioxide in an air tight vessel and then stored at different temperatures. At varying intervals of time the butter was examined for the development of flavors. This experiment was repeated about forty times. Bacteriological examination of the butter was also made in some of the experiments. The results from one of such experiments are given in table 4.

The bacterial counts in table 4 are very irregular. A partial explanation for this probably lies in the fact that the bacteria are unevenly distributed in the butter and that an accurate counting of bacteria in solid dairy products is difficult. But in spite of this variation the results indicate that the storing of butter in carbon dioxide tends to suppress the bacterial activity in the butter. At warmer temperature this suppression is of small duration and the bacteria soon increase in numbers. At colder temperature this suppression is more pronounced. The yeasts showed a slight increase which was largely controlled by the temperature.

There was one outstanding difference between the butter stored in carbon dioxide and that stored in the air, namely, that the butter stored in carbon dioxide was always free from the molds.

The keeping quality of the butter was also affected by the storage in the carbon dioxide. In most cases the butter stored in carbon dioxide kept a little longer before off flavors began to appear. This effect was also greatly influenced by the temperature and probably by some other causes. At a warm temperature the treated butter did not keep very much longer, while at a cold temperature it was a matter of several days and at times several weeks. However, this prolongation of the keeping quality of the butter was not permanent, for in time the treated butter also developed off flavors. The flavors in most cases were differ-

TABLE 4

*Bacterial, yeast and mold counts of the butter stored in carbon dioxide at different temperatures*

| AGE OF BUTTER | NUMBER OF BACTERIA PER GRAM | | NUMBER OF YEASTS AND MOLDS PER GRAM | |
|---|---|---|---|---|
| | Butter in $CO_2$ | Butter in air | Butter in $CO_2$ | Butter in air |
| Butter kept at 70°F. | | | | |
| 5 days —1/30/24 | 590,000 | 870,000 | 130,000 | 140,000 |
| 10 days —2/ 6/24 | 4,800,000 | 3,100,000 | 585,000 | 181,000 |
| 2 weeks—2/15/24 | 4,410,000 | 4,110,000 | 1,278,000 | 2,340,000 |
| 4 weeks—2/25/24 | 2,240,000 | 14,500,000 | 1,250,000 | 2,630,000 |
| 5 weeks—3/ 3/24 | 2,370.000 | 9,300,000 | 1,170,000 | 2,270,000 |
| 7 weeks—3/17/24 | 7,500,000 | 4,000,000 | 2,020,000 | 2,250,000 |
| Butter kept at 60°F. | | | | |
| 5 days —1/30/24 | 290,000 | 159,000 | 300 | 200 |
| 10 days —2/ 6/24 | 87,000 | 78,000 | 18,800 | 2,200 |
| 2 weeks—2/15/24 | 278.000 | 374,000 | 39,600 | 101,700 |
| 4 weeks—2/25/24 | 118.000 | 166,000 | 44,000 | 154,000 |
| 5 weeks—3/ 3/24 | 500,000 | 300,000 | | |
| 7 weeks—3/17/24 | | | 412,000 | 514,000 |
| 10 weeks—4/14/24 | 290,000 | 1,600,000 | 520,000 | 460,000* |
| Butter kept at 50°F. | | | | |
| 5 days —1/30/24 | 180,000 | 297.000 | 200 | 500 |
| 10 days —2/ 6/24 | 151,000 | 429,000 | 200 | 14,900 |
| 2 weeks—2/15/24 | 56,500 | 176,000 | 300 | 1,100 |
| 4 weeks—2/25/24 | 44,900 | 480,000 | 200 | 100,300 |
| 5 weeks—3/ 4/24 | 49,000 | 386,000 | 400 | 121,000 |
| 7 weeks—3/17/24 | 480,000 | 835,000 | 14,300 | |
| 10 weeks—4/14/24 | 108,000 | 1,600,000 | 290,000 | 229,000* |
| Butter kept at 40°F. | | | | |
| 5 days —1/30/24 | 445,000 | 250,000 | 200 | 300 |
| 10 days —2/ 6/24 | 68,000 | 40,000 | | 300 |
| 2 weeks—2/15/24 | 57,700 | 63,000 | | 200 |
| 4 weeks—2/25/24 | 582,000 | 98,600 | 800 | 24,200 |
| 5 weeks—3/ 4/24 | 110,000 | 90,000 | 300 | 50,000 |
| 7 weeks—3/17/24 | 440,000 | 730,000 | 800 | |
| 14 weeks—5/ 8/24 | 500,000 | 1,090,000 | 1,100 | 540,000 |
| Butter kept at 32°F. | | | | |
| 5 days 1/30/24 | 441,000 | 345,000 | 700 | 300 |
| 10 days 2/ 6/24 | 220,000 | 211,000 | 200 | |
| 4 weeks—2/26/24 | 176,000 | 178,000 | 250 | 450 |
| 7 weeks—3/17/24 | 83,000 | 156,000 | 650 | 5,900 |
| 14 weeks—5/ 8/24 | 299,000 | 1,400,000 | 48,000 | 98,000 |

* Many molds.

ent and occasionally even more objectionable than in the uncarbonated butter.

## SUMMARY AND CONCLUSIONS

The carbonation of sweet cream as done in these experiments tended to suppress certain types of bacteria but did not hinder other types of bacteria from their multipliction. This effect of the carbonation caused a delay in the souring of the cream. The length of time of this delay depended on the temperature at which the cream was held and probably also on the amount and on the kind of the initial bacterial contamination. At room temperature the delay was a matter of about two hours while at a temperature of 35°F. it was a matter of several days.

When the carbon dioxide was applied at churning time by charging the cream in the churn and by replacing the air above the cream in the churn by the gas, no significant benefit appeared to be derived therefrom. The gas caused the butter when fresh to taste sourish and this taste tended to cover the taste due to the neutralizer used in the cream. The molds were not inhibited by this treatment. Both the laboratory as well as the commercial creamery experiments showed that this method of application of carbon dioxide did not measurably affect the germ life in the butter nor did it cause the butter to have better flavors to to keep longer.

When the carbon dioxide was applied by storing the butter in the gas in an air tight container, it completely inhibited the development of the molds in the butter. It also prolonged the keeping quality of the butter when held at cold temperature. However, in time the butter stored in carbon dioxide also developed undesirable flavors.

The result of these experiments indicate that the carbonation of the cream intended for butter, or the carbonation of the butter during churning operation does not result in any special benefit to justify the use of carbon dioxide in this manner.

Hunziker (11), reported that the carbonation of butter as done commercially—that is, when applied at churning time—is of no special value to the butter industry.

Rettger, Winslow and Smith (12) found that the carbonation of ice cream as done commercially has no appreciable affect on the germ life or on the vitamin in the ice cream.

Prucha, Brannon and Ambrose (10) reported that they could find no significant effect of carbonation of ice cream on the germ life therein.

Such results are to be expected when the available information on the influence of carbon dioxide on germ life is carefully examined.

The carbonation of beverages is successful because the beverages are carbonated and are kept in air tight containers in this gas, under pressure. Donald Jones and Maclean recommended a pressure of 70 pounds. If these drinks were carbonated at the time they are prepared and then left in unsedled containers the beneficial effect of carbonation would disappear. Our results from experiments on milk kept under pressure indicate that much higher pressure is necessary in case of dairy products to completely inhibit the bacterial activities in them.

All the evidence from these studies point to the conclusion that in order to obtain any appreciable benefits from the carbonation of the butter, it is necessary to store the butter in the atmosphere of carbon dioxide in an air tight container. Whether the benefits derived from such treatment are of any economic value will have to be demonstrated commercially on a large scale.

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# SOME STUDIES IN REDUCING THE STABILITY OF BUTTERFAT EMULSIONS*

H. A. RUEHE AND B. A. STIRITZ

*Department of Dairy Husbandry, University of Illinois, Urbana, Illinois*

The purpose of this study was to determine some of the factors which tend to stabilize emulsions of butterfat in buttermilk obtained by the churning of cream; and, if possible, to develop methods which would at least partially destroy the stability of the emulsion and render a more complete churning of the cream with a lower loss of fat in the buttermilk.

It is common knowledge that butterfat exists in cream in the form of minute globules, held in more or less stable "oil-in-water" emulsion. The churning of cream consists primarily in subjecting it to violent agitation, so that the globules of fat are forced in contact with each other. If this is done at the proper temperature the globules will break through the thin film of milk serum, separating them from one another and coalesce, until finally these adhering globules form butter granules which are large enough to be readily separated from the fluid buttermilk in which they float. In other words, the mechanical churning breaks down the oil-in-water emulsion and forms butter, which is in reality a water in oil emulsion. This change, however, is not complete, and a certain amount of fat remains in a rather stable oil in water emulsion, which constitutes a fat loss to the butter manufacturer.

There are numerous factors affecting the losses of fat in buttermilk and many of these, such as correct temperature, fullness of the churn, and acidity of the cream are carefully watched and controlled in order to keep the fat losses down to a minimum. Nevertheless, there is a considerable loss. The average fat content of fifty-seven samples of buttermilk secured from various

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* Received for publication May 10, 1925.

creameries in Illinois, was 0.914 per cent. Under existing conditions this fat which is lost seems to be in such a stable emulsion as to be unchurnable.

There are, no doubt, several factors responsible for this extreme stability. The components of milk or cream existing normally in colloidal solution act as stabilizing agents. Much of the cream received in butter manufacturing plants has a high acid content, and in order to properly prepare this cream for pasteurization, a portion of the acid is reduced by the addition of solutions of various kinds of alkali. Those most commonly used are calcium lime, calcium-magnesium lime, sodium carbonate, and sodium bicarbonate. There is no doubt that this neutralizing process is not always carried out properly and that at times, a certain amount of saponification takes place. Donnon (1) has shown by his work that soap is an excellent emulsifying agent for fat. Other investigators (2) have also verified the results obtained by Donnon.

There are also certain compounds formed by the action of these alkalies upon the proteins of the cream and these products are excellent emulsifying agents, and no doubt, are important factors in stabilizing the emulsion of fat in buttermilk. The influence of electrical charges in stabilizing colloidol emulsions, may also be a factor.

Hardy (3) has suggested that when colloidal partiles are coagulated under the influence of electrolytes, the fat globules absorb oppositely charged ions, so that their charges become neutralized, and at the iso-electric point, stability is lost (2). Ellis (4) found that the rate of coagulation of the oil globules was practically independent of the interfacial tension, depending only on the electrical potential at the surface of the globules. On adding HCl to an oil-in-water emulsion, the interfacial tension did not vary within the limits of experimental error; while the stability decreased enormously as the acid was added (2).

The conclusions of Ellis seem somewhat in disagreement with the work of Bredig (5), who reports that there is a decrease of surface tension with coagulation. However, Ellis considers the electrical factors of far more importance than the effect of the interfacial tension.

Early investigators considered viscosity as an important factor in stabilizing oil-in-water emulsions. At the present time, however, many investigators in this field do not consider viscosity as a cause for emulsification, but rather that viscosity aids emulsification solely by virture of the of the hindrance offered to the coalescence of the dispersed fat globules.

According to Clayton (2), the hydration theory of emulsions as proposed by Fisher (6) is subject to several objections. This hydration theory considers it necessary to have a certain minimum amount of water present in order to emulsify oil in a hydrated colloid such as casein. Furthermore, it is considered that if water is present in an amount greater than that used in hydrating the colloid, no stable emulsion is possible.

This theory does not seem applicable to the problem under consideration, since in buttermilk water is present in an amount very much in excess of that necessary to hydrate the casein; and yet, there is a stable emulsion of fat in buttermilk.

In addition to the factors pointed out above, there is also a possibility that considerable fat is lost in buttermilk, due either to mechanical entrapping of some of the minute globules of fat in casein particles, or by the adsorption of fat globules by these components of the buttermilk.

The relative importance of this theory as compared with the other theories mentioned above could readily be ascertained by separating buttermilk into its two component parts—curd and whey, and determining the fat content of each. This procedure was followed. Special attention, however, was given to investigating the electrolyte theory of stability of fat emulsions, the substances used as electrolytes, were HCl, NaCl, Th $(NO_3)_4$, oleic acid and other chemicals. Since butter is a food, nothing could be used that would impair its wholesomeness, nor destroy the possibilities of the buttermilk being used for feeding purposes.

## METHOD OF PROCEDURE

To determine the proportion of whey and curd in a sample of buttermilk, the following method was used: A heavy glass tube was filled with a known weight of well mixed buttermilk and centrifuged for seventy-five minutes at a speed of 1200 r.p.m. (a pressure of 40.6 pounds per square inch was generated). This force was sufficient to cause a complete separation of the curd and whey into distinct layers, which could be separated and analyzed. The amount of fat in the curd and whey was determined by multiplying their weights and tests, respectively, and from this the relative amounts of fats were calculated, using the total in the buttermilk as 100 per cent.

The fat tests on all samples were determined by using the Mojonnier tester. For buttermilk and whey, a 10-gram sample was measured into an extraction flask, 1.5 cc. of ammonia water was added and the flask shaken. Sufficient alcohol was added to bring the contents up to the top of the small neck of the bulb and the flask again shaken. Twenty-five cubic centimeters of petroleum ether were added and the total contents thoroughly mixed. The flask was centrifuged in order to separate the ether solution of fat, and this was decanted into a weighed dish. This extraction was repeated using 15 cc. of ethyl and petroleum ether, respectively. The second extraction was added to the dish containing the first, and the solution evaporated to dryness, being heated to a constant weight at 135°F. under a 25-inch vacuum. The dishes were cooled, weighed, and the percentage of fat calculated.

The fat in the curd was determined as follows: 2 to 3 grams of curd were weighed into a Mojonnier butter boat, and then placed into an extraction flask. Ten cubic centimeters of hot water were added, and after the contents were mixed thoroughly, the procedure was carried out as described for buttermilk and whey.

Forty-nine samples of buttermilk were tested to determine the proportion of curd and whey and their fat contents. These samples were secured from cream which had been neutralized with the following substances: rock lime, Mitchell lime, Alwood lime, soda ash, a combination of Alwood lime and soda ash, and an unneutralized check sample. The strength of each of the neutralizers was approximately 15 per cent, except in the case of soda ash when a 10 per cent solution was used. Samples of buttermilk were taken from the same churn at the start and near the end of the draining, in order to determine if there was any variation in the fat content.

Working on the theory that hydrochloric acid or sodium chloride would neutralize the electrical charge and break the emulsion of the fat, churnings were made in the following ways: A large batch of cream was neutralized, pasteurized and cooled to churning temperature. It was divided into three lots of equal size and churned as nearly the same time as possible, the only variation in each churning being the treatment given the cream in the churn. Lot 1 was churned with no treatment and was used as the check churning. One per cent salt was added to lot 2 and to lot 3, sufficient hydrochloric acid was added to increase the acidity 0.05 per cent, calculated as lactic acid. The acid and salt were added to the cream as it went into the churn.

Churnings were repeated in exactly the same manner except that a combination of 1 per cent salt and sufficient acid to increase the acidity of the cream 0.05 per cent, calculated as lactic acid, were used.

Later, the salt and acid method was carried out on a commercial scale. The cream was prepared by being neutralized with Mitchell lime and pasteurized, and immediately before churning, the batch was divided into two equal parts of 1900 pounds. These lots were churned simultaneously in 1000-pound Dual churns, driven by the same shaft and motor. One churning was untreated and was used as a check. To the other, 490 cc. of 37 per cent hydrochloric acid and 19 pounds of salt were added. The time to churn, temperature, etc., were recorded on each.

The work was repeated in another creamery where Alwood lime was used as a neutralizer for the cream, and where three churnings of 2600 pounds were secured from the same vat of cream. One churning was used as the check, to the second, 25 cc. of hydrochloric acid and 1 pound of salt were added for each 100 pounds of cream and the third churning was treated with varying amounts of acid and salt to determine the effect of varying concentrations of these substances.

Another series of thirty-three experiments were repeated in the second plant, substituting Peerless hydrated lime for Alwood lime. The method of procedure was exactly the same as in the previous experiments except in this set of experiments, two pails of cold water were added to some of the churnings when the butter began to break to determine the effect upon the emulsified fat.

Acidity tests were made upon the butter in order to determine the effect of the HCl upon the acidity of the finished product. The following method, described by Bouska (8), was used. Nine grams of butter were melted to the consistency of thick cream. To this were added 20 cc. of a mixture of 100 parts ether and 25 parts of alcohol. This was titrated with N/20 NaOH, using phenolphthalein as an indicator and the acidity computed.

In some churnings, other chemicals were used in the same way as the HCl and NaCl in an attempt to destroy the emulsion. Di-sodium phosphate, sodium citrate, 10 per cent solution of oleic acid and thorium nitrate were added to cream for this purpose.

## DISCUSSION OF RESULTS

The largest part of all buttermilk samples was whey, as was shown by separating the curd and the whey by centrifugal force. Table 1 shows the maximum percentage to be 90 per cent; the minimum, 79.7 per cent; and the average of 79 samples, 82.5 per cent.

There is no set rule as to where the fat in buttermilk is found. The greatest portion may be held in the curd or in the whey, depending upon whether or not the cream is neutralized and upon the neutralizer used, and also upon other conditions affecting losses in churning, such as acidity, temperature, speed of churn, time held, etc. Buttermilk from cream, which was neutralized with soda ash, contained approximately three-fourths of the fat in the whey, whereas, that from unneutralized cream, contained three-fourths of the fat in the curd. The other neuralizers varied between these two extremes. The average of all buttermilk samples was 47.8 per cent of the fat in the whey and 52.2 per cent in the curd.

The analyses of samples of buttermilk taken at the start and at the end of draining showed no variation in the fat content. In ten experiments, the maximum variation between two samples of buttermilk from the same churning was 0.018 per cent; the minimum, 0.004 per cent; and the average, 0.009 per cent. A buttermilk sample taken at any time during draining will be representative providing no fat granules get into the buttermilk. Probably, the best time to take a sample is just after the first buttermilk has passed off (which washes away any cream from the churn gate) and while the butter granules are still floating in the churn.

TABLE 1

*The relative amount of whey contained in buttermilk obtained from cream untreated and treated with HCl and NaCl*

| TREATMENT | NUMBER OF SAMPLES | MAXIMUM WHEY | MINIMUM WHEY | AVERAGE WHEY |
|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* |
| None | 49 | 88.9 | 80.9 | 82.3 |
| Salt | 11 | 90.0 | 79.7 | 85.5 |
| HCl | 11 | 86.0 | 69.6 | 80.3 |
| HCl and salt | 8 | 85.7 | 77.5 | 81.9 |
| Total or average | 79 | 87.6 | 78.9 | 82.5 |

TABLE 2

*The relative amount of fat contained in the whey and curd of buttermilk from cream unneutralized and neutralized*

| NEUTRALIZER | FAT IN WHEY | | | FAT IN CURD | | |
|---|---|---|---|---|---|---|
| | Maximum | Minimum | Average | Maximum | Minimum | Average |
| Rock lime (CaO) | 68.1 | 30.3 | 54.2 | 69.7 | 31.9 | 45.8 |
| Mitchell lime | 84.0 | 32.4 | 46.1 | 67.6 | 16.0 | 53.9 |
| Alwood lime (Mg. lime) | 63.4 | 38.3 | 48.3 | 61.7 | 36.6 | 51.7 |
| Soda ash | 84.4 | 51.0 | 71.8 | 49.0 | 15.6 | 28.2 |
| Alwood lime and soda ash | 59.8 | 22.3 | 26.3 | 77.7 | 40.2 | 58.8 |
| None | 27.6 | 25.1 | 26.3 | 74.9 | 72.4 | 73.7 |
| Average of all | 64.5 | 33.2 | 47.8 | 66.8 | 35.5 | 52.2 |

Table 3 shows the results of churning different lots of cream, each divided into three parts and treated as indicated. Both salt and acid when used separately had some effects in liberating the fat in the curd and whey.

The results tend to show that churning with acid sets free a larger amount of fat from the curd than from the whey, since a larger percentage of the total fat was found in the whey when acid was used, than in the check churning. The tendency of the salt was to free more from the curd than did the acid. The results showed a greater amount of the fat was held in the whey with salt than with acid or the check churning.

TABLE 3

*Use of NaCl and HCl in churning*

| NEUTRALIZER | TREATMENT | BUTTERMILK TEST | RELATIVE AMOUNT OF FAT | | AVERAGE REDUCTION IN TEST OF BUTTERMILK DUE TO TREATMENT |
|---|---|---|---|---|---|
| | | | In whey | In curd | |
| Lime | None | 1.048 | 54.1 | 45.9 | |
| | Salt | 0.858 | 77.4 | 32.6 | 0.190 |
| | HCl | 0.720 | 70.7 | 39.3 | 0.328 |
| Soda ash | None | 0.782 | 71.8 | 28.2 | |
| | Salt | 0.563 | 77.5 | 22.5 | 0.219 |
| | HCl | 0.484 | 75.0 | 25.0 | 0.298 |
| Alwood lime | None | 0.832 | 48.3 | 51.7 | |
| | Salt | 0.636 | 63.9 | 36.1 | 0.196 |
| | HCl | 0.602 | 59.5 | 40.5 | 0.230 |
| Mitchell lime | None | 1.709 | 46.2 | 53.8 | |
| | Salt | 1.162 | 71.9 | 28.1 | 0.547 |
| | HCl | 0.882 | 59.2 | 40.8 | 0.827 |
| Alwood lime and soda ash | None | 0.849 | 41.2 | 58.8 | |
| | Salt | 0.667 | 57.7 | 42.3 | 0.182 |
| | HCl | 0.503 | 49.0 | 51.0 | 0.346 |
| All neutralizer average | None | 1.044 | 52.3 | 47.7 | |
| | Salt | 0.777 | 65.7 | 34.3 | 0.267 |
| | HCl | 0.638 | 62.7 | 37.3 | 0.406 |

Table 4 shows the results obtained when a combination of acid and salt were used. The addition of this combination tends to free the fat held in the curd more than does either when used separately. It also tends to liberate more fat from the curd than it does from the whey. In each case, the larger percentage of fat was found in the whey than in the check or untreated sample.

The average reduction of fat losses in buttermilk, when salt was added, was 0.267 per cent as compared with the check churning and it was 0.406 per cent when acid was used. When a combination of both was used, the reduction was 0.480 per cent.

TABLE 4

*Results of churnings to which both HCl and salt were added*

| NEUTRALIZER | TREATMENT | BUTTERMILK TEST | FAT | | FAT REDUCTION |
|---|---|---|---|---|---|
| | | | Whey | Curd | |
| Alwood lime | None | 1.010 | 53.2 | 46.8 | |
| | Salt and acid | 0.456 | 86.0 | 14.0 | 0.554 |
| Mitchell lime | None | 1.221 | 47.8 | 52.2 | |
| | Salt and acid | 0.704 | 79.6 | 30.4 | 0.517 |
| Alwood lime and soda ash | None | 0.721 | 48.3 | 51.7 | |
| | Salt and acid | 0.352 | 69.0 | 31.0 | 0.369 |
| All neutralizers averaged | None | 0.984 | 49.8 | 50.2 | |
| | Salt and acid | 0.504 | 78.2 | 21.8 | 0.480 |

TABLE 5

*Results obtained by the use of salt and HCl in commercial plant where Mitchell lime was used for neutralizing the cream*

| TREATMENT | | BUTTERMILK TEST | FAT | | FAT REDUCTION |
|---|---|---|---|---|---|
| | | | Whey | Curd | |
| None | Maximum | 1.012 | 32.4 | 67.6 | |
| | Minimum | 0.644 | 46.1 | 53.9 | |
| | Average | 0.828 | 39.2 | 60.8 | |
| Salt and HCl | Maximum | 0.720 | 60.0 | 40.0 | 0.292 |
| | Minimum | 0.232 | 82.3 | 17.7 | 0.412 |
| | Average | 0.476 | 71.1 | 28.9 | 0.352 |

Tables 5 to 7 give the results obtained in commercial plants. In the first two plants, acid and salt were used. One per cent of salt was added to the cream and sufficient 37 per cent hydrochloric acid to increase the acidity of the cream 0.05 per cent, calculated as lactic acid. Table 7 gives the results obtained on a second visit to the plant in which the results of table 6 were secured. The only variation was the substitution of Peerless hydrated lime for Alwood Lime.

TABLE 6

*Results obtained by the use of NaCl and HCl in commercial plant where Alwood lime was used for neutralizing*

| TREATMENT | | BUTTERMILK TEST | FAT | | FAT REDUCTION |
|---|---|---|---|---|---|
| | | | Whey | Curd | |
| None | Maximum | 0.758 | 48.0 | 52.0 | |
| | Minimum | 0.422 | 23.9 | 76.1 | |
| | Average | 0.598 | 34.1 | 65.9 | |
| Salt and HCl | Maximum | 0.540 | 85.0 | 15.0 | 0.218 |
| | Minimum | 0.282 | 31.9 | 63.1 | 0.140 |
| | Average | 0.443 | 55.2 | 44.8 | 0.155 |

TABLE 7

*Results obtained by the use of salt and HCl in a commercial plant where Peerless hydrated lime was used*

| TREATMENT | | BUTTERMILK TEST | FAT | | FAT REDUCTION |
|---|---|---|---|---|---|
| | | | Whey | Curd | |
| | | | *per cent* | *per cent* | |
| None | Maximum | 1.014 | 30.2 | 69.8 | |
| | Minimum | 0.706 | 19.0 | 81.0 | |
| | Average | 0.780 | 24.4 | 75.6 | |
| Salt and acid | Maximum | 0.762 | 34.5 | 65.5 | 0.252 |
| | Minimum | 0.522 | 20.2 | 79.8 | 0.144 |
| | Average | 0.636 | 27.6 | 72.4 | 0.184 |
| $H^2O$ added as cream breaks* | Maximum | 0.694 | 25.8 | 74.2 | 0.178 |
| | Minimum | 0.660 | 21.0 | 79.0 | 0.046 |
| | Average | 0.677 | 23.4 | 76.6 | 0.080 |
| Water, acid and salt* | Maximum | 0.734 | 31.2 | 68.8 | 0.280 |
| | Minimum | 0.562 | 26.2 | 73.8 | 0.144 |
| | Average | 0.621 | 28.8 | 71.2 | 0.159 |

* Two 12-quart pails of cold water were added to the churn when the cream started to break.

The results obtained in the commercial plants were very similar to those obtained in preliminary work carried on in the laboratory. The fat content of the curd was lowered by the acid and salt. The results obtained when Peerless lime was used, were somewhat different from those obtained with the other lime. When the results in table 7 are compared to those in tables 5 and 6, it will be noticed that fat reductions were secured in each, however, there was an outstanding difference in the fat content of the curd in the treated as compared with the check churning. Neutralizers seem to have a tendency to cause such variations. When Peerless lime was used, the fat reduction came from both the curd and whey in practically the same proportion. On the other hand, when either Mitchell or Alwood lime was used, the reduction occurred more in the curd than the whey. The effect that the various neutralizers have in forming a compound which stabilizes the emulsion is another problem yet to be solved.

The adding of water to the churn as the cream started to break did not lower materially the per cent of fat in the buttermilk. Some special emulsions can be destroyed by dilution, however, our results do not show this to be true with buttermilk. The reduction secured was due to dilution, since the adding of two buckets of water increased the volume of buttermilk. The combination of water, salt and acid did not decrease the fat loss any more than did the salt and acid, except that caused by dilution of the buttermilk.

By comparing table 5 with tables 6 and 7, it can be seen that in the one plant where a more exhaustive churning was secured with no treatment, the average reduction was less when salt and acid were used than in the plant with the larger fat losses. The first plant has an average fat loss of 0.828 per cent and the second 0.689 per cent. With the use of acid and salt, the percentage fat in the buttermilk was reduced to 0.476 per cent in the first plant, and 0.539 per cent in the second. The minimum for each plant was nearly the same. This tends to show that there was a certain amount of emulsified fat in the whey, and some held by the curd, which was not churnable under the conditions used.

The other chemicals, which were used, did not give results which were at all promising. The average reduction in the test of the buttermilk secured with di-sodium phosphate was 0.06 per cent. Results obtained with sodium citrate showed only an average reduction of 0.02 per cent. These results come within the limits of experimental error.

In one set of experiments, 5 cc. of a 10 per cent solution of oleic acid was added to 2 liters of cream in addition to the salt and HCl. These were compared with untreated churnings. This treatment reduced the fat in the buttermilk to 0.48 per cent. Further trials with this treatment were not attempted because the finished product had a decided off flavor of oleic acid which would eliminate its use.

TABLE 8

*Use of thorium nitrate*

| LOT NUMBER | TREATMENT | AMOUNT OF THORIUM NITRATE USED | THORIUM NITRATE IN LOT OF CREAM | REDUCTION OR INCREASE DUE TO THORIUM NITRATE |
|---|---|---|---|---|
| | | *grams* | *per cent* | |
| | Unneutralized | 4.62 | 0.019 | −0.13 |
| 2 | Lime used | 9.46 | 0.044 | +0.71 |
| 3 | Lime used | 4.62 | 0.018 | +0.14 |

TABLE 9

*Effect of the use of HCl on the acidity of the butter*

| LOT NUMBER | TREATMENT | ACIDITY OF BUTTER |
|---|---|---|
| 1 | None | 0.1405 |
| | HCl | 0.1385 |
| 2 | None | 0.1525 |
| | HCl | 0.1550 |

Clayton (2), states that thorium nitrate will invert an emulsion. A series of experiments were made using this material. The results obtained are given in table 8.

The results obtained with thorium nitrate do not indicate that it would prove of value in destroying the fat emulsion in buttermilk.

The use of HCl did not seem to have any effect upon the total acidity of the butter, as is shown in table 9. Apparently the HCl is removed in the buttermilk and wash water.

The 1 per cent of salt used is largely removed with the buttermilk and wash water, although a small amount is retained by the butter. Analyses of the butter showed that it contained from 0.2 to 0.3 per cent salt, the average being 0.25 per cent.

The quality of the butter as determined by three judges, was not affected by the salt and acid treatment. One of the judges was consistent in scoring the treated samples about one-half point higher than the untreated butter. This was possibly due to the fact that the treated sample did contain a small amount of salt, whereas the check sample was unsalted.

## CONCLUSIONS

1. Seventy-nine samples of buttermilk which had been subjected to centrifugal force and separated into the components, whey and curd, gave an average whey content of 82.5 per cent.
2. The fat contained in buttermilk is partly held in stable emulsion in the whey, and partly in the curd, either by adsorption or mechanical entrapping. The relative amounts held by the curd and whey are variable and dependent upon numerous factors such as the kind of neutralizer used, acidity and temperature of the cream, speed of churn, etc.
3. The addition of 1 per cent of NaCl to the cream before churning will tend to decrease fat losses in the buttermilk. A small amount of salt, 0.2 to 0.3 per cent, is incorporated in the butter.
4. The addition of HCl to the cream before churning is more affective than NaCl in reducing the fat loss in buttermilk. The HCl added to the cream apparently does not increase the total acidity of the butter.
5. The use of the combination of NaCl and HCl is more affective in reducing fat losses in churning than either of these when used alone.

In this investigation it was not possible to reduce the fat content of buttermilk from neutralized cream, below an average of approximately 0.5 per cent.

6. The use of di-sodium phosphate, sodium citrate, or thorium nitrate did not prove effective in decreasing the fat content of buttermilk. Oleic acid, because of its deteriorating effect on the quality of the butter, was eliminated as being an unsatisfactory substance to use.

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# A STUDY OF THE ORGANISMS CAUSING THICKENING OF SWEETENED CONDENSED MILK*

PAUL A. DOWNS

*Department of Dairy Husbandry, University of Nebraska, Lincoln, Nebraska*

Sweetened condensed milk, as found in the market, usually flows freely when removed from the can. The contents have a color closely resembling that of cream, and possess a pleasing odor and taste. Occasionally, however, one finds a can with the contents so thick that they will not flow without vigorous stirring, or, perhaps, are even solid.

Two different kinds of thickening that may cause this condition have been observed. One of these, commonly called heat or age thickening, apparently causes a change only in the viscosity of the milk, the taste and odor remaining normal. The other kind of thickening, which is considered in this paper, produces different conditions. In the early stages, the milk may show a normal body having clots of thickened milk throughout. Sometimes the milk may be so thick that it will barely flow. In later stages it may be solid, so that it is possible to invert the can without the loss of its contents. As the degree of thickening increases, acidity and a characteristic fruity odor develops, as well as a disagreeable taste. When vigorously stirred, the milk thins slightly, but it returns to its original thickness after standing. When a portion of the milk is diluted with water and heated, a separation of the curd takes place. By this test it can be distinguished from heat or age thickened milk.

This kind of thickening commonly referred to as bacterial thickening has been found to occur most commonly in late spring or summer or in shipments to tropical countries.

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* A thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Cornell University, Ithaca, New York. Received for publication April 16, 1925.

## LITERATURE

Probably the first to report such a spoilage was Greig-Smith (1) who described a type of thickening in which he found large numbers of coccus forms of bacteria. He isolated a form with which he was able to again produce thickening in condensed milk. The organism he described as being a coccus was distinguished from Micrococcus pyogenes albus only by its being non-pathogenic to mice. The thermal death point in milk was found to be 63°C. for ten minutes.

From the fact that he was able to isolate the organism from samples of condensed milk manufactured in Switzerland and Norway that did not show signs of thickening in any way, he concluded that the organism was of widespread occurrence.

Hunziker (2) discusses "thickened and cheesy condensed milk" also, and points out that the conditions can be prevented by the use of large amounts of sugar in manufacture.

Rice and Downs (3) also report this type of thickening from the manufacturer's point of view, especially in regard to standardization and temperature influences.

## EXPERIMENTAL

It was the purpose of this work to study the organisms causing bacterial thickening, to determine whether or not they were common in condensed milk as found on the market, and if possible to determine their distribution.

In carrying out the study, organisms were isolated from a sample of thickened condensed milk which had been returned by the trade and from factory contamination. A large number of organisms were also isolated from samples of condensed, powdered and market milk as well as from the air. These, together with a group of coccus forms obtained from several collections of type cultures, were studied. Organisms isolated from thirty-six samples of fluid milk and others isolated from eleven samples of commercial condensed milk were also studied.

The organisms obtained were tested for their thickening ability in sweetened condensed milk by the procedure given below.

Those giving positive results were studied further to determine their cultural and biochemical characteristics so that they might be classified.

### *Procedure used in the thickening tests*

Each lot of sweetened condensed milk used for the thickening tests was analyzed and standardized with sterile water to 71 per cent total solids. Lots 1, 2 and 3 were then put into sterile quart milk bottles, pasteurized at 145° to 150°F. for thirty minutes and cooled to 50°F. All other lots of milk used were pasteurized in a shot-gun can at a temperature of 150° to 155°F. for one hour. This change was made because the checks on lot 3 showed that some of the thickening organisms had survived the treatment given it.

After being cooled, 170 grams of each sample of milk was put into sterile glass-stoppered bottles.

When the organisms to be tested had been invigorated in 1 per cent sucrose broth for several days, 5 cc. of the culture was added to a bottle of pasteurized condensed milk. It was mixed well by vigorous shaking, and was then incubated at 30°C. for six weeks. With the 5 cc. of broth added, the total solids of the test milk was approximately 70 per cent.

Check bottles of each lot of milk were kept, and in every instance, with the exception of lot 3, the pasteurized milk gave negative results. Check bottles of 70 and 71 per cent unpasteurized milk all thickened except those in lot 2. This would seem to indicate that the thickening organism is often present in commercial condensed milk, and that when the solids are lowered thickening will take place. The lots of milk just described were used in all thickening tests as carried out with cultures of each series except where otherwise stated.

## ISOLATION OF THE THICKENING ORGANISM

### *a. From thickened samples of commercial milk*

The first attempt to isolate the thickening organism was made on commercial sweetened condensed milk which had thickened and had developed the fruity odor and the disagreeable taste already mentioned. The acidity shown by titration was found to be high, and bacterial examination showed many thousands of coccus forms to the gram. Further study showed that the milk contained a practically pure culture of these cocci. The colonies appeared to be of two kinds, one white and the other straw color. On agar slants, they produced a filiform, white-to-transparent growth. When they were inoculated into litmus milk, it became acidified and a curd formed which contracted and slowly peptonized, leaving a pink, acid whey.

These cultures were inoculated into sweetened condensed milk containing approximately 70 per cent total solids. After the milk had been incubated at 30°C. for a few weeks, the samples thickened and developed the characteristic fruity odor and high acidity. It was found that this thickened milk, when mixed with water, went into solution very slowly. When a portion of the diluted milk was heated, coagulation took place and the curd separated.

From this data it seemed apparent that the organism isolated was similar to the one described by Greig-Smith. It was evident that the high titratable acidity which accompanied the thickening and the acid peptonization of the litmus milk tubes would be valuable determinative tests which could be used in the study.

### *b. From factory contamination*

As no other samples of thickened milk were available for this study, an experiment was planned with the hope that a thickened sample of milk could be produced. It was quite evident that if the thermal death point of the organism was 63°C., as reported by Greig-Smith, none of the thickening organisms would survive the condensing process. Therefore, they would have to get into the milk while it was being handled, after it had left the condensing pan. This, together with the fact that the usual practice of the manufacturers to prevent this type of spoilage is to raise the percentage of sugar in the milk, led to the following assumptions: First, that factory contaminations, such as deposits from the packing boxes of pasteurizers or of cooling vats, and water from equipment and floor, might contain the organism. Second, that if high sugar content stopped its growth, normal sweetened condensed milk, diluted slightly, ought to allow the organism, if present, to grow.

On the basis of these two assumptions, a quantity of fresh condensed milk containing 73.5 per cent total solids and 43 per cent sucrose was procured. This was divided into four parts, which respectively were diluted to approximately 73, 70, 65 and 60 per cent total solids by the addition of 5 cc. of factory contamination and sterile water in the required amounts. The factory contamination was a mixture of material removed from the packing box of the pasteurizer and of water taken from a broken place in the floor of the department creamery during its operation. To this was added a small amount of slime from an old skim-milk cheese. In mixing the samples, dishes were used which had previously been exposed to the air of the laboratory for several days. The standardized and inoculated mixtures were placed in sterile bottles. These were incubated at 30°C. for eight weeks, and were examined at the end of two, three and eight weeks.

These examinations showed that the samples containing 65 and 60 per cent total solids developed thickening after two weeks of incubation. The 70-per cent sample showed thickening at three weeks, and the 73-per cent sample at eight weeks. In every instance the thickening was accompanied by the fruity odor and the high acidity.

Bacterial examination showed that all samples contained large numbers of coccus forms. Ten cultures were isolated from the four samples which will hereafter be referred to as series S.

Several tests were made with the ten cultures of this series and the results are recorded in table 1. While each culture does not appear in every lot of milk, at least duplicate determinations were made. The check sample for each lot of milk is recorded at the bottom of the table for reference. A plus sign indicates that thickening or fruity odor was present, and a minus sign shows its absence. Where a question mark is found slight changes were observed, but not enough to warrant their being called positive.

From these results it is evident that the only cultures showing consistent thickening, together with fruity odor and high acidity were S-4 and S-8. A cultural and morphological study of these two cultures showed that they were similar to those which had been isolated from the sample of thickened condensed milk, as reported under heading (*a*).

Where the thickening organism came from cannot be stated, as the sources of inoculation were several in number. The results experienced with the different batches of milk used for the thickening tests showed that the thickening organism might have been present in the original condensed milk. It might also have come from any of the other sources of contamination, or from the air.

TABLE 1

*Results of thickening tests with cultures of series S*

(Incubation period, six weeks at 30°C.)

| CULTURE | MILK USED | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lot 1 | | | Lot 2 | | | Lot 3 | | | Lot 4 | | |
| | Body | Odor | Lactic acid | Body | Odor | Lactic acid | Body | Odor | Lactic acid | Body | Odor | Lactic acid |
| | | | *per cent* | | | *per cent* | | | *per cent* | | | *per cent* |
| S-1 | – | – | 0.334 | | | | – | – | 0.545 | ? | ? | 0.670 |
| S-2 | | | | – | – | 0.416 | | | | – | – | 0.395 |
| S-3 | | | | – | – | 0.307 | | | | – | – | 0.390 |
| S-4 | + | + | 0.503 | | | | + | + | 0.900 | + | + | 0.950 |
| S-5 | | | | – | – | 0.335 | | | | ? | | 0.600 |
| S-6 | | | | | | | – | – | 0.562 | – | – | 0.380 |
| S-7 | | | | ? | – | 0.439 | | | | – | ? | 0.760 |
| S-8 | | | | + | + | 0.510 | + | + | 0.990 | + | + | 0.960 |
| S-9 | | | | ? | – | 0.460 | | | | – | ? | 0.830 |
| S-10 | | | | – | – | 0.364 | | | | – | – | 0.500 |
| S-check | – | – | 0.326 | – | – | 0.363 | + | + | 0.645 | – | – | 0.425 |

As this experiment added two more cultures to the group of thickening organisms, further thickening tests were run with a group of organisms isolated from miscellaneous material.

### *c. From miscellaneous material*

A group of twenty-three coccus forms, series R were isolated from miscellaneous material as described below.

Cultures R-1, R-7, R-8, R-9, R-17, R-18, R-19 and R-20 were isolated from condensed milk samples. Cultures R-2, R-3, R-4, R-5, and R-6 were from powdered milk. Samples of rancid condensed milk furnished cultures R-14, R-15, and R-16. Cultures R-10, R-11 and R-12 were obtained from market milk plates while R-21 and R-22 came from air plates from out doors.

TABLE 2

*Results of thickening tests with cultures of series R*

(Incubation period, six weeks at 30°C.)

| CULTURE | MILK USED | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lot 1 | | | Lot 2 | | | Lot 3 | | | Lot 4 | | | Lot 5 | | |
| | Body | Odor | Lactic acid | Body | Odor | Lactic acid | Body | Odor | Lactic acid | Body | Odor | Lactic acid | Body | Odor | Lactic acid |
| | | | *per cent* | | | *per cent* | | | *per cent* | | | *per cent* | | | *per cent* |
| R-check | – | – | 0.326 | – | – | 0.363 | + | + | 0.645 | – | – | 0.425 | – | – | 0.324 |
| R-1 | | | | | | | | | | – | – | 0.500 | | | |
| R-2 | + | + | 0.585 | | | | + | + | 1.050 | + | + | 0.760 | | | |
| R-3 | | | | | | | – | – | 0.525 | – | – | 0.385 | | | |
| R-4 | | | | | | | – | – | 0.520 | ? | ? | 0.780 | | | |
| R-5 | | | | | | | | | | – | – | 0.460 | | | |
| R-6 | | | | | | | | | | – | – | 0.490 | | | |
| R-7 | | | | – | – | 0.396 | | | | | | | | | |
| R-8 | | | | ? | | 0.495 | | | | | | | | | |
| R-9 | – | – | 0.347 | – | – | 0.307 | – | – | 0.480 | – | – | 0.500 | | | |
| R-10 | – | – | 0.355 | ? | | 0.440 | | | | | | | | | |
| R-11 | | | | | | | | | | – | – | 0.500 | | | |
| R-12 | | | | | | | | | | – | – | 0.430 | | | |
| R-13 | | | | – | – | 0.306 | – | – | 0.765 | – | – | 0.490 | | | |
| R-14 | + | + | 0.502 | | | | | | | – | – | 0.430 | | | |
| R-15 | – | – | 0.310 | | | | | | | | | | | | |
| R-16 | | | | | | | – | – | 0.535 | – | – | 0.470 | | | |
| R-17 | – | ? | 0.462 | | | | | | | – | ? | 0.550 | | | |
| R-18 | – | – | 0.320 | | | | | | | ? | ? | 0.590 | | | |
| R-19 | – | – | 0.325 | | | | | | | – | – | 0.450 | | | |
| R-20 | | | | | | | | | | – | – | 0.500 | | | |
| R-21 | – | – | 0.330 | | | | | | | – | – | 0.550 | | | |
| R-22 | | | | | | | | | | | | | + | + | 0.459 |
| R-23 | | | | | | | | | | | | | + | + | 0.396 |

These organisms were tested for thickening power by the same procedure used in studying the cultures of series S, and the results appear in table 2.

A study of the results obtained with series R shows that culture R-2 consistently produced thickening in three tests. Culture R-14 gave positive results in the first test, but failed to do so in the second trial. Cultures R-22 and R-23 were only tested once, as they were isolated almost at the completion of the work, when there was no time for further tests.

TABLE 3

*List of pure cultures, obtained from various collections, used in thickening tests*

| SAMPLE | NAME OF CULTURE | NUMBER | COLLECTION |
|---|---|---|---|
| A-1 | Staphylococcus aureus | 4 | American Museum of Natural History |
| A-2 | Staphylococcus aurantiacus | 348 | |
| A-3 | Staphylococcus epidermidus | 25a | |
| A-4 | Staphylococcus ureae | 464a | |
| A-5 | Staphylococcus candidus | 49a | |
| A-6 | Staphylococcus tetragenus | 209 | |
| A-7 | Staphylococcus candicans | 526 | |
| A-8 | Staphylococcus pyogenes aureus | 4 | |
| A-9 | Staphylococcus pyogenes albus | 163 | |
| A-10 | Micrococcus flavus | 272 | |
| A-11 | Micrococcus citreus | 466 | |
| A-12 | Sarcina lutia | 208 | |
| A-13 | Mixed cultures | 4 + 263 | |
| A-14 | Mixed cultures | 4 + 209 | |
| A-15 | Micrococcus lactis varians | 129 | W. M. Esten |
| A-16 | Micrococcus lactis varians | 130 | |
| A-17 | Staphylococcus creamoris viscosi | | Lister Institute |
| A-18 | Staphylococcus pyogenes albus I | | |
| A-19 | Staphylococcus pyogenes albus | | Army Medical School |
| A-20 | Staphylococcus pyogenes albus | 121 | W. M. Esten |

Considering R-2, R-22, and R-23 as positive thickeners, these cultures were studied and found to be similar to the other thickening organisms isolated. Culture R-2, when grown in milk, showed a slight difference in its rate of peptonization from that shown by the other cultures, but otherwise it was the same.

It is of interest to note that the source of R-2 was a sample of powdered milk, while R-22 and R-23 came from the air.

As seven cultures of the thickening organism had thus far been isolated it was decided to test next a series of cultures of known origin. Pure cultures were accordingly obtained from various collections, as listed in table 3.

TABLE 4

*Results of thickening tests with cultures of series A*

(Incubation period, six weeks at 30°C.)

| CULTURE | MILK USED | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lot 1 | | | Lot 2 | | | Lot 3 | | | Lot 4 | | | Lot 5 | | |
| | Body | Odor | Lactic acid | Body | Odor | Lactic acid | Body | Odor | Lactic acid | Body | Odor | Lactic acid | Body | Odor | Lactic acid |
| | | | *per cent* | | | *per cent* | | | *per cent* | | | *per cent* | | | *per cent* |
| A-check | — | — | 0.326 | — | — | 0.363 | + | + | 0.645 | — | — | 0.424 | — | — | 0.324 |
| A-1 | — | — | 0.312 | — | — | 0.437 | | | | — | — | 0.400 | | | |
| A-2 | | | | | | | — | — | 0.590 | — | — | 0.400 | | | |
| A-3 | — | — | 0.468 | | | | | | | — | — | 0.460 | | | |
| A-4 | | | | | | | — | — | 0.500 | — | — | 0.440 | | | |
| A-5 | | | | | | | | | | — | — | 0.450 | | | |
| A-6 | — | — | 0.321 | — | — | 0.425 | | | | — | — | 0.390 | | | |
| A-7 | — | — | 0.350 | | | | — | — | 0.460 | — | — | 0.490 | | | |
| A-8 | | | | | | | | | | — | — | 0.400 | | | |
| A-9 | — | — | 0.369 | | | | | | | — | — | 0.950 | | | |
| A-10 | | | | — | — | 0.406 | | | | ? | ? | 0.695 | | | |
| A-11 | | | | — | — | 0.408 | | | | ? | ? | 0.670 | | | |
| A-12 | | | | | | | — | — | 0.515 | — | — | 0.500 | | | |
| A-13 | | | | — | — | 0.574 | | | | — | — | 0.740 | | | |
| A-14 | | | | ? | — | 0.380 | | | | — | — | 0.470 | | | |
| A-15 | | | | | | | | | | | | | — | — | 0.405 |
| A-16 | | | | | | | | | | | | | — | — | 0.324 |
| A-17 | | | | | | | | | | | | | — | — | |
| A-18 | | | | | | | | | | | | | — | — | 0.288 |
| A-19 | | | | | | | | | | | | | — | — | |
| A-20 | | | | | | | | | | | | | — | — | |

These cultures were tested for thickening power as the other cultures had been. Several cultures of Staphylococcus pyogenes albus were obtained and included in this series, which will be referred to as series A. Beside testing pure cultures, mixed cultures were used in A-13 and A-14 to determine whether or not there was any associative action.

In studying the results shown in table 4 it will be noticed that in not a single instance was thickening produced. The thickening organism may resemble Micrococcus pyogenes albus, as stated by Greig-Smith (1). But the results already given would indicate that this organism is not able to produce thickening in sweetened condensed milk. The writer assumes that Micrococcus pyogenes albus referred to by Greig-Smith was the organism described by Rosenbach, and with which the name Staphylococcus pyogenes albus is used synonymously.

### *d. From fluid milk*

Considering fluid milk as a possible source of the thickening organism, several samples of fluid milk were studied. It at once became evident that some cultural characteristic would have to be used to distinguish the possible thickeners from the other organisms.

As the acid peptonization of litmus milk seemed to be a characteristic by which the thickening organisms could be distinguished from other milk organisms, the following procedure was used to isolate them. Milk samples were plated on standard nutrient agar, and incubated from two to four days at 30°C. All the colonies on a plate or on part of a plate were picked out and inoculated into litmus milk tubes. These were incubated at 30°C. for ten days.

In a preliminary study it was found that over 11 per cent of the cultures isolated from samples of fluid milk showed acid peptonization. It at once became evident from these results that if many samples of milk were to be examined and the thickening power of all the members of the peptonizing group tested, some other method would have to be devised than that used in the first part of the work, because of the time and the space required for these determinations.

After experimenting with various different methods, the following procedure was adopted: A sample of commercial sweetened condensed milk was obtained and examined. If it appeared to be normal and the acidity as shown by titration was 0.3 per cent lactic acid or below, it was accepted for use. The condensed milk was transferred to a sterile bottle, weighed,

TABLE 5

*Occurrence of thickening organisms in fluid milk*

Thirty-six samples

| SAMPLE NUMBER | PLATE COUNT PER GRAM | CULTURES ISOLATED | ACID PEPTONIZERS FOUND | THICKENERS FOUND | |
|---|---|---|---|---|---|
| | | | | 50 per cent | 70 per cent |
| | *number* | *number* | *number* | *number* | *number* |
| 15 | 9,600 | 20 | 2 | 0 | 0 |
| 16 | 10,600 | 15 | 0 | 0 | 0 |
| 18 | 2,200 | 11 | 0 | 0 | 0 |
| 19 | 5,200 | 16 | 2 | 0 | 0 |
| 20 | 4,700 | 25 | 2 | 1 | 0 |
| 21 | 14,700 | 18 | 15 | 1 | 0 |
| 22 | 7,000 | 11 | 0 | 0 | 0 |
| 23 | 10,000 | 9 | 0 | 0 | 0 |
| 24 | 24,000 | 16 | 1 | 0 | 0 |
| 25 | 115,000 | 15 | 0 | 0 | 0 |
| 26 | 113,000 | 15 | 11 | 0 | 0 |
| 27 | 34,000 | 13 | 0 | 0 | 0 |
| 28 | 4,200 | 15 | 2 | 0 | 0 |
| 29 | 6,200 | 12 | 7 | 0 | 0 |
| 30 | 52,000 | 14 | 3 | 0 | 0 |
| 31 | 37,000 | 11 | 0 | 0 | 0 |
| 32 | 27,000 | 13 | 3 | 0 | 0 |
| 33 | 13,000 | 15 | 3 | 0 | 0 |
| 34 | 380,000 | 17 | 11 | 0 | 0 |
| 35 | 10,000 | 14 | 1 | 1 | 0 |
| 36 | 12,100 | 14 | 4 | 0 | 0 |
| 37 | 6,700 | 12 | 6 | 0 | 0 |
| 38 | 52,000 | 20 | 1 | 0 | 0 |
| 53 | | 15 | 0 | 0 | 0 |
| 57 | | 17 | 0 | 0 | 0 |
| 34-x | 182,000 | 12 | 1 | 0 | 0 |
| 35-x | 11,000 | 13 | 1 | 0 | 0 |
| 36-x | 16,000 | 14 | 0 | 0 | 0 |
| 37-x | 13,900 | 17 | 0 | 0 | 0 |
| 38-x | 16,900 | 13 | 0 | 0 | 0 |
| 39-x | 80,000 | 9 | 0 | 0 | 0 |
| 40-x | 156,000 | 16 | 0 | 0 | 0 |
| 41-x | 98,000 | 16 | 1 | 0 | 0 |
| 42-x | | 14 | 0 | 0 | 0 |
| 43-x | | 17 | 3 | 1 | 0 |
| 46-x | | 9 | 0 | 0 | 0 |
| Totals.................. | | 523 | 80 (15.2%) | 4 | 0 |

and standardized to 50 per cent total solids with sterile water. It was then thoroughly mixed by shaking, and was transferred to sterile test tubes plugged with cotton. Ten cubic centimeters of the condensed milk was placed in each tube.

These tubes were immediately inoculated with approximately 0.5 cc. of a litmus-milk culture of the organism to be tested. The tubes were then incubated at 30°C. for two days in a position similar to that used in slanting agar. Cultures of the thickening organism gave a solid coagulation of the milk, which retained its slant form even when vigorously shaken and jarred. This type of coagulation was considered a positive test.

By this method it was possible to examine cultures obtained from numerous samples of milk from individual patrons as well as the mixed milk in the creamery and city milk samples.

While studying this source, thirty-six samples of fluid milk were studied with results as shown in table 5. Of the 523 cultures isolated, 80 cultures classed as acid peptonizers were inoculated, into 50 per cent, sweetened condensed milk and incubated as previously described. Of this number only four cultures produced thickening. These cultures were then tested in 70 per cent sweetened condensed milk in which they all failed to produce thickening.

Although the thickening organism itself was not found in fluid milk, it is quite evident that organisms that are similar do occur and that these organisms can only be distinguished from the thickeners by the thickening test.

### *e. From commercial canned milk*

The first study of commercial canned milk was made on ten samples of common brands. The bacterial content per gram, as shown by the plate method, varied greatly, ranging from 30 to 868,000 per gram. Of 20 cultures of cocci isolated, all, with the exception of three cultures from one sample, were inactive in litmus milk after incubation for two weeks. The three exceptions showed slight coagulation and then very slight peptonization. These results gave the impression that the samples did not contain the thickening organisms. Further study, however brought out the fact that probably a large percentage of the cultures isolated from these samples were the thickening organisms.

Subsequent to the first study, eleven more samples were obtained and studied. The samples were bought on the market, from the local stores. The cans were disinfected with 5 per cent carbolic acid and dried over a flame before being opened. The contents were then transferred to sterile bottles, and stored in the refrigerator. The percentages of solids and of sucrose were determined, and the condition of the sample was observed. The results are recorded in table 6.

TABLE 6

*Showing the brands used and the condition of the commercial samples at the time of purchase*

| SAMPLE | BRAND | PERCENTAGE COMPOSITION | | | CONDITION OF SAMPLE | | |
|---|---|---|---|---|---|---|---|
| | | Total solids | Sucrose | | | | |
| | | | Total | In water | Body | Taste | Odor |
| | | *per cent* | *per cent* | *per cent* | | | |
| C-1 | Thrift | | | | O. K. | Slightly rancid | Rancid |
| C-2 | Dairymen's League | | | | O. K. | O. K. | O. K. |
| C-3 | A and P | | | | O. K. | O. K. | O. K. |
| C-4 | Eagle | 72.52 | 42.21 | 60.56 | O. K. | O. K. | O. K. |
| C-5 | Eagle | 72.75 | 40.26 | 59.63 | O.K. | O. K. | O. K. |
| C-6 | Thrift | 73.99 | 44.85 | 63.29 | O. K. | Tallowy | Tallowy |
| C-7 | A and P | 74.23 | 41.46 | 61.69 | O. K. | O. K. | O. K. |
| C-8 | A and P | 74.54 | 44.22 | 63.43 | O. K. | O. K. | O. K. |
| C-9 | Eagle | 72.94 | 42.22 | 61.02 | O. K. | O. K. | O. K. |
| C-10 | A and P | 74.45 | 43.80 | 63.15 | Heavy | Old | O. K. |
| C-11 | A and P | 74.45 | 43.61 | 63.00 | O. K. | O. K. | O. K. |

A bacterial count was made by the plate method. Ten-gram samples were used, and dilutions used were 100, 1000 and 10,000. Standard nutrient agar was the medium employed, and the plates were incubated for four days at 30°C. The acidity was determined by titrating with tenth normal sodium hydroxide, a ten-gram sample diluted with 90 cc. of distilled water. The percentage of acid was reported in terms of lactic acid.

A portion of a plate from each sample was selected, and all the colonies on that portion were picked and inoculated into both litmus milk and agar slants. Notes were kept concerning the types of colonies that predominated, and it was observed that in most of the samples there appeared colonies that closely resembled the typical colony of the thickening organism as did the agar stroke cultures. However, the litmus-milk cultures did not show acid peptonization.

Meanwhile, a portion of each of the original samples had been incubated at 30°C., in order to show the effect of this temperature. It was observed that at the end of ten days samples C-3 and C-10 had thickened to the point of solidity and had developed the characteristic fruity odor. A study of the litmus milk cultures from sample C-3 showed 19 inert reactions out of 20 cultures, while those from C-10 showed 8 inert out of 9 cultures. This observation, together with the fact that these same cultures produced colonies and growths on agar slants that resembled that of the thickening organisms, showed that for some reason the thickening organism, when isolated from samples of condensed milk, does not always produce acid peptonization in litmus milk.

In order to determine whether or not this might be a different organism from the one previously studied, further observations were made. A number of cultures were invigorated in a 1 per cent sucrose-and-lactose broth. The cultures were transferred daily for two weeks, and then were inoculated into litmus milk. When these cultures were incubated, acid peptonization was produced, as in the cultures of the thickening organism originally studied.

It was evident that the peptonization of litmus milk could not be used as a means of identification on organisms that had been isolated from commercial samples of sweetened condensed milk. The identification of the organism from commercial samples was therefore based on the typical colony produced on agar plates, on the typical growth of the agar slant, and on the ability of the organism to thicken 50 per cent, sweetened condensed milk. These tests were further substantiated by inoculating representative cultures from each sample into 70 per cent condensed milk to test their thickening properties.

Using the same procedure as before, the original samples were again plated and cultures isolated, with the results shown in table 7. Thickening organisms were found in all but four of the samples as purchased. In some of the samples, the organism was present in a practically pure culture. The fact that the body and the acidity of the milk were normal would indicate that the organisms had gained entrance in large numbers during its manufacture. This view is further strengthened by the fact that the samples in which no thickening organisms were found at this time were also very low in bacteria count.

TABLE 7

*Occurrence of thickening organisms in commercial samples at the time of purchase*

| SAMPLE | LACTIC ACID | BACTERIA COUNT PER GRAM | CULTURES ISOLATED | THICKENING ORGANISM FOUND | PERCENTAGE OF ALL CULTURES THAT WERE THICKENERS |
|---|---|---|---|---|---|
| | *per cent* | *number* | *number* | *number* | *per cent* |
| C-1 | 0.550 | 215 | 12 | 0 | 0 |
| C-2 | 0.392 | 351,000 | 10 | 9 | 90 |
| C-3 | 0.401 | 32,400 | 12 | 12 | 100 |
| C-4 | 0.391 | 9,500 | 9 | 8 | 89 |
| C-5 | 0.333 | 140 | 12 | 0 | 0 |
| C-6 | 0.327 | 150 | 14 | 0 | 0 |
| C-7 | 0.333 | 6,300 | 13 | 2 | 16 |
| C-8 | 0.303 | 124,000 | 14 | 9 | 64 |
| C-9 | 0.303 | 30 | 11 | 0 | 0 |
| C-10 | 0.354 | 43,000 | 15 | 14 | 93 |
| C-11 | 0.366 | 5,200 | 13 | 6 | 46 |

Sixty cultures of the thickening organisms were isolated from the samples as they were purchased. After the samples had been held six weeks at 30°C., another examination was made, and seventy-three cultures of the thickening organism were isolated. A part of each sample was diluted to 70 per cent solids, and was held at 30°C. for six weeks. Eighty-three cultures of the thickening organism were then isolated from them. From each of the thirty three samples studied in this way, a representative culture was taken and inoculated into 70 per cent milk, as has been done in the other experiments. The results of this test are given in table 8. More cultures were isolated from the

TABLE 8

*Results of thickening test on 33 representative organisms isolated from commercial samples*

| SAMPLE | CULTURE | BODY | ODOR | LACTIC ACID |
|---|---|---|---|---|
| When purchased | | | | |
| | | | | *per cent* |
| 1 | C-1 | – | – | |
| 2 | C-2 | + | + | 0.531 |
| 3 | C-3 | + | + | 0.585 |
| 4 | C-4 | + | + | 0.540 |
| 5 | C-5 | – | – | 0.315 |
| 6 | C-6 | – | – | 0.297 |
| 7 | C-7 | + | + | 0.585 |
| 8 | C-8 | + | + | 0.396 |
| 9 | C-9 | – | – | 0.301 |
| 10 | C-10 | + | + | 0.565 |
| 11 | C-11 | + | + | 0.576 |
| After being held at 30°C. for six weeks | | | | |
| 1 | I-C-1 | Culture lost | | |
| 2 | I-C-2 | Culture lost | | |
| 3 | I-C-3 | + | + | 0.605 |
| 4 | I-C-4 | ? | ? | 0.387 |
| 5 | I-C-5 | + | + | 0.693 |
| 6 | I-C-6 | + | + | 0.694 |
| 7 | I-C-7 | + | + | 0.630 |
| 8 | I-C-8 | + | + | 0.675 |
| 9 | I-C-9 | + | + | 0.602 |
| 10 | I-C-10 | + | + | 0.630 |
| 11 | I-C-11 | + | + | 0.522 |
| Standardized to 70 per cent solids and held at 30°C. for six weeks | | | | |
| 1 | I-C-1-70 | – | – | |
| 2 | I-C-2-70 | + | + | 0.639 |
| 3 | I-C-3-70 | + | + | 0.666 |
| 4 | I-C-4-70 | + | + | 0.441 |
| 5 | I-C-5-70 | + | + | 0.630 |
| 6 | I-C-6-70 | + | + | 0.621 |
| 7 | I-C-7-70 | + | + | 0.324 |
| 8 | I-C-8-70 | + | + | 0.558 |
| 9 | I-C-9-70 | – | – | |
| 10 | I-C-10-70 | + | + | 0.657 |
| 11 | I-C-11-70 | + | + | 0.600 |

same samples after nine weeks of incubation, making a total of 476 cultures, of which 311 were reported as thickeners.

To ascertain what degree of accuracy could be expected in these determinations, tabulations were made. Thirty cultures were classified, according to observations made on the type of colony and on the growth on agar slants, as follows: 24 positive, 2 questionable, and 4 negative.

When they were tested in 70 per cent milk, the following results were obtained: 23 positive, 1 questionable, and 6 negative. It is obvious from these results, therefore, that the figures given are fairly accurate.

### *Summary of isolation experiments*

Isolation of the thickening organism was attempted from five sources: thickened condensed milk (*a*); factory contamination (*b*); miscellaneous material (*c*); fluid milk (*d*); and commercial samples of condensed milk (*e*).

A total of 1054 cultures were studied, and 318 cultures were reported as thickening organisms. A total of 284 thickening tests were run in 70 per cent, sweetened condensed milk. So many cultures were obtained from experiment E that only representative cultures were studied from each sample. These samples were studied in the condition in which they were found at the time of their purchase, and again after being held at 30°C. for six weeks, and, finally, after they had been diluted to 70 per cent total solids and held for six weeks at 30°C. In this way the thickening organism was found in all of the samples except C-1. The thirty-two cultures of the thickening organism recorded in table 9 were considered as representative, and were subsequently studied further.

If we consider the isolation experiments from the view point of the possible source of the organism in the sweetened condensed milk, we obtain the results recorded in table 10.

Of the entire 318 cultures of the thickening organism isolated, 313 came from commercial samples of condensed milk and from the original sample of thickened milk. The rest comprised two cultures isolated from factory contamination, one from powdered milk, and two from the air.

TABLE 9

*List of the thirty-two cultures of thickening organisms studied and their source of isolation*

| CULTURE | ISOLATED FROM |
|---|---|
| T-1 | Samples of thickened condensed milk |
| T-2 | |
| S-4 | Factory contamination |
| S-8 | |
| R-2 | Powdered milk |
| R-22 | Outdoor air |
| R-23 | |
| C-2 | Commercial samples as purchased |
| C-3 | |
| C-4 | |
| C-7 | |
| C-8 | |
| C-10 | |
| C-11 | |
| I-C-3 | Commercial samples incubated 6 weeks |
| I-C-4 | |
| I-C-5 | |
| I-C-6 | |
| I-C-7 | |
| I-C-8 | |
| I-C-9 | |
| I-C-10 | |
| I-C-11 | |
| I-C-2-70 | Commercial samples diluted to 70 per cent solids and incubated |
| I-C-3-70 | |
| I-C-4-70 | |
| I-C-5-70 | |
| I-C-6-70 | |
| I-C-7-70 | |
| I-C-8-70 | |
| I-C-10-70 | |
| I-C-11-70 | |

That condensed milk acts as a differential medium for this organism is very evident. The only specific place where thickening organisms were found was the air. It is quite possible, indeed, that the organisms in the factory contaminations came also from the air.

If the air is a source of contamination, it is easy to see how condensed milk might become contaminated after manufacture, especially in factories where sanitary conditions are not of the best. If this were true, one would expect to find some correlation between the bacterial content of sweetened condensed milk and the presence of the thickening organism, and a study of these two factors, as shown in table 7, shows that this is the case.

TABLE 10

*Summary of the isolation experiments*

| EXPERIMENT | SAMPLES STUDIED | CULTURES STUDIED | THICKENING ORGANISMS FOUND |
|---|---|---|---|
| | *number* | *number* | *number* |
| Thickened condensed milk (*a*) | 1 | 2 | 2 |
| Factory contamination (*b*) | 4 | 10 | 2 |
| Miscellaneous material (*c*) | | 42 | 3 |
| Fluid milk (*d*) | 46 | 523 | 0 |
| Commercial samples (*e*) | 11 (55) | 476 | 311 |

## DESCRIPTION OF THICKENING ORGANISMS

The thirty-two cultures reported in table 9 were further studied to determine whether they were all the same species of organism.

A complete study of the thirty-two cultures was made, as outlined on the descriptive chart endorsed by the committee on pure-culture study of the Society of American Bacteriologists, in their recent reports (4).

The method here reported for comparison is the brief characterization as given on the chart but with a few additions. The cultural features in litmus milk were believed to be especially important in this work, and therefore were included. The fermentation of glycerine was also added. These additions made a total of seventeen instead of nine figures. The explanation of the numerical values is to be found in the key.

*Key to the brief characterization of cultures*

*Microscopic features:*
  Form: 1, streptococci; 2, diplococci; 3, micrococci; 4, sarcinae; 5, rods; 6, commas; 7, spirals; 8, branched rods; 9, filamentous.
  Spores: 1, central; 2, polar; 3, absent.
  Flagella: 1, peritrichic; 2, polar; 3, absent.
  Gram stain: 1, positive; 2, negative.
*Miscellaneous biochemical reactions:*
  Pathogenicity, and so forth: 1, for man; 2, for animals; 3, for plants; 4, parasitic but not pathogenic; 5, saprophytic; 6, autotrophic.
  Relation to oxygen; 1, strict aerobe; 2, facultative anaerobe; 3, strict anaerobe.
  Gelatin liquefaction: 1, positive; 2, negative.
  In nitrate media: 1, nitrite and gas; 2, nitrite but no gas; 3, neither nitrite nor gas.
  Chromogenesis: 1, fluorescent; 2, violet; 3, blue; 4, green; 5, yellow; 6, orange; 7, red; 8, brown; 9, pink; 0, none.
*Carbohydrate reactions:*
  Diastatic action: 1, positive; 2, negative.
  From dextrose: 1, acid and gas; 2, acid without gas; 3, no acid.
  From lactose: 1, acid and gas; 2, acid without gas; 3, no acid.
  From sucrose: 1, acid and gas; 2, acid without gas; 3, no acid.
  From glycerine: 1, acid and gas; 2, acid without gas; 3, no acid.
*Cultural features in milk:*
  Acid: 1, sufficient for curdling; 2, insufficient for curdling; 3, no acid.
  Rennet curd: 1, present; 2, absent.
  Peptonization: 1, present; 2, absent.

Results of the study of thirty-two cultures of thickeners are recorded in table 11. These results show that there are two, quite distinct groups of organisms. Most of the organisms isolated from the commercial samples of condensed milk neither fermented lactose nor reduced nitrates. They were also inactive in litmus milk. Their ability to ferment lactose could be restored, however, by continued invigoration in lactose broth; but their ability to reduce nitrates was never definitely demonstrated.

It is also interesting to note that neither culture I-C-4 nor culture I-C-7-70 fermented sucrose or lactose, and thus they produced little or no acid in the thickening test, as shown in table 8. However, both of these cultures thickened milk. This might indicate that the thickening process was not due to acid but to a rennin-like enzyme. This possibility is further confirmed by the fact that I-C-4 gave a rennet curd in litmus milk.

Just why there should be this difference in the organisms isolated from commercial milk is hard to explain. It would seem logical that, if the organism uses sucrose instead of lactose when growing in commercial milk, as shown by Rice and Downs (3), its lactose-fermenting enzyme would gradually become dormant. If this is true, when the organism is removed from the presence of

TABLE 11

*The brief characterization number of the thirty-two cultures of the thickening organisms studied*

| CULTURE | MICROSCOPIC FEATURES | BIOCHEMICAL REACTIONS | CARBOHYDRATE REACTIONS | LITMUS-MILK REACTIONS |
|---|---|---|---|---|
| T-1 | 3–3–3–1 | 4–2–1– –0 | 2–2–2–2– | 1–2–1 |
| T-2 | 3–3–3–1 | 4–2–1– –5 | 2–2–2–2– | 1–2–1 |
| S-4 | 3–3–3–1 | 4–2–1–2–0 | 2–2–2–2–2 | 1–2–1 |
| S-8 | 3–3–3–1 | 4–2–1–2–0 | 2–2–2–2–2 | 1–2–1 |
| R-2 | 3–3–3–1 | 4–2–1–2–0 | 2–2–2–2–2 | 1–2–1 |
| R-22 | 3–3–3–1 | 4–2–1–2–0 | 2–2–2–2–2 | 1–2–1 |
| R-23 | 3–3–3–1 | 4–2–1–2–0 | 2–2–2–2–2 | 1–2–1 |
| C-2 | 3–3–3–1 | 4–1–1–3–0 | 2–2–2–2–2 | 1–2–2 |
| C-3 | 3–3–3–1 | 4–2–1–3–5 | 2–2–3–2–2 | 3–2–2 |
| C-4 | 3–3–3–1 | 4–2–1–3–0 | 2–2–3–2–2– | 3–2–2 |
| C-7 | 3–3–3–1 | 4–2–2–3–0 | 2–2–3–2–2 | 3–2–2 |
| C-8 | 3–3–3–1 | 4–1–1–3–6 | 2–2–3–2–2 | 2–2–2 |
| C-10 | 3–3–3–1 | 4–2–1–3–5 | 2–2–3–2–2 | 3–2–2 |
| C-11 | 3–3–3–1 | 4–2–2–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-3 | 3–3–3–1 | 4–2–1–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-4 | 3–3–3–1 | 4–2–2–3–0 | 2–2–3–3–2 | 3–1–2 |
| I-C-5 | 3–3–3–1 | 4–2–1–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-6 | 3–3–3–1 | 4–2–2–3–0 | 2–2–3–2–2 | 3–2–2 |
| I-C-7 | 3–3–3–1 | 4–2–2–3–0 | 2–2–3–2–2 | 3–2–2 |
| I-C-8 | 3–3–3–1 | 4–2–1–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-9 | 3–3–3–1 | 4–2–1–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-10 | 3–3–3–1 | 4–2–2–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-11 | 3–3–3–1 | 4–2–2–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-2-70 | 3–3–3–1 | 4–2–1–2–0 | 2–2–2–2–2 | 1–2–2 |
| I-C-3-70 | 3–3–3–1 | 4–2–2–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-4-70 | 3–3–3–1 | 4–2–2–2–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-5-70 | 3–3–3–1 | 4–2–2–3–0 | 2–2–3–2–2 | 3–2–2 |
| I-C-6-70 | 3–3–3–1 | 4–2–1–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-7-70 | 3–3–3–1 | 4–2–1–2–0 | 2–2–3–3–2 | 3–2–2 |
| I-C-8-70 | 3–3–3–1 | 4–2–1–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-10-70 | 3–3–3–1 | 4–2–1–3–5 | 2–2–3–2–2 | 3–2–2 |
| I-C-11-70 | 3–3–3–1 | 4–2–2–3–5 | 2–2–3–2–2 | 3–2–2 |

sucrose, which is its only source of energy, it should appear to be a different organism, and so it does. However, continued growth in the presence of lactose, with no other source of energy present, should cause the lactose-fermenting enzyme gradually to become active again. In fact, this seems to be what happens. But why the nitrates are not reduced to nitrites is a subject for further study.

### *Description of variety A*

Index number: 3331-42120-22222-121.

*Morphology.* Cultures studied from growth on plain-agar slants, after incubation for two days at 30°C., appear as cocci. The individuals appear as single cells, pairs, and irregular clusters. The size of the organism varies from 0.4 to 0.5 microns in diameter. The organisms stain readily with all common stains, and no capsule is demonstrated. The gram is positive.

*Temperature relations.* Growth on agar slants and in broth attains its maximum at 30°C. There is a slight growth at 20°C., and at 37°C. growth is a trifle less than at 30°C.

*Agar-stroke culture.* Four days at 30°C. produce a moderate, filiform-to-beaded, raised, and glistening growth. The surface is slightly contoured, with its edges raised above the level of the center. The growth is translucent, without any chromogenesis or odor, and generally viscid in consistency.

*Agar colonies.* The surface colonies incubated four days at 30°C. show moderate growth, circular colonies, and a smooth surface with convex elevation. The edges appear to be beveled, and sometimes the center is depressed. The edge is entire, and the internal structure is amorphous. The diameter of the colonies varies from 0.75 to 1.5 mm. and the color, from translucent to pale white.

*Subsurface colonies.* The subsurface colonies are lens shaped, and approximately 0.5 mm. in length. They have a tendency to appear slightly yellowish or straw colored, but they become whitish when growth breaks through to the surface.

*Gelatin stab.* After fourteen days at 20°C. in plain gelatin, growth appears along the line of the stab. This growth is beaded at the bottom, and infundibuliform. Its liquefaction begins in four days. After sixty days it appears as stratiform to the depth of about 1 mm. This description was found to be variable in the same culture, depending on the age of the culture.

*Gelatin colonies at 20°C. (seven days).* Punctiform colonies appear, with slight depression around them.

*Litmus milk at 30°C.* Type a: An acid curd appears after two days, and a shrinking of the curd, and a reduction of the litmus takes place after about four days. As more time passes, the curd contracts and the whey becomes pink and gives an acid reaction to methyl red. Slight peptonization appears after seven days, but it never becomes complete, even when held a year.

Type b: This type is the same as type a, except that the curd begins to peptonize after two days, instead of contracting, and the form of the curd disappears into a mass of granular substance at the bottom of the tube.

*Fermentation.* Acid is produced without gas from fructose, dextrose, lactose, sucrose, and glycerine. It first appears after one day at 30°C. After from two to four days the broth gives a yellow color with brom phenol blue, indicating an approximate pH value of 3.5 to 3. No acid is produced from raffinose, adonite, dulcite, dextrin, or inulin.

*Oxygen requirements.* When the organism grows in dextrose, lactose, sucrose and glycerine broth, it produces a heavy cloud without any gas in the closed arm as well as in the bowl of the fermentation tube.

*Nutrient broth.* On nutrient broth no surface growth appears except a very slight ring growth at the end of from two to four days. A heavy cloud, with a compact and viscid sediment, results from continued incubation.

*Nitrate reduction.* Nitrates are reduced, in four days, to nitrites, when grown on beef-extract nitrate agar.

*Other tests.* When cultures were grown in peptone water for seven days ammonia and indol were produced. No diastatic action is shown when the colonies are grown on starch-agar plates. No hydrogen sulphide is produced.

*Thermal death point.* A broth culture held at 64°C. for ten minutes was killed, but was not killed at 63°C. for ten minutes.

### *Description of variety B (as isolated from commercial samples of condensed milk)*

Index number: 3331-42235-22322-322.

*Morphology.* The morphology of variety B is the same as that of variety A.

*Agar-stroke culture.* The growth is the same as that of variety A, except that the growth is more rapid. Good growth is obtained in from four to six hours at 30°C. Some cultures show lemon-yellow chromogenesis, both white and yellow sometimes appearing on the same slant.

*Agar colonies.* These are the same as those of variety A, except for their chromogenesis.

*Gelatin stab.* Liquefaction is produced, as in variety A, but it is slower and sometimes absent.

*Gelatin colonies at 20°C. (seven days).* Punctiform colonies appear and liquefaction is questionable.

*Litmus milk at 30°C.* No change takes place in seven days. In two cultures slight acidity was noted, and coagulation was found after fourteen days.

*Fermentation.* Acid is produced, without gas, in dextrose, sucrose, and glycerine. Lactose was not fermented except when slight acidity appeared in the litmus milk.

*Oxygen requirements.* The growth produces a heavy cloud in closed arm with sucrose, dextrose and glycerine broth.

*Nitrate reduction.* Nitrates are not reduced to nitrites.

*Ammonia production.* A small amount of ammonia is produced in peptone water. The production of indol is questionable. No diastatic action or hydrogen sulphide is produced.

These data seem to further confirm the previous belief that the organisms found were all variations of one type of organism. Probably the Staphylococcus pyogenes albus I of the Lister Institute resembles the particular organism in question as closely as do any of the organisms studied. However, this culture did not thicken condensed milk. The question of whether the thickening organism might not lose its ability to thicken milk after a period of time might be raised. While none of the cultures have been carried in stock for a great length of time, some of them were used for more than three years. These cultures were therefore again tested for thickening at the completion of the investigation, and were found to be as vigorous as ever.

## CONCLUSIONS

The group of organisms found to thicken sweetened condensed milk were all of one general type, of which two distinct varieties were found.

Variety A was obtained from one sample of thickened milk, from factory contamination, from air plates, and from powdered milk. This organism was identical with a culture of Staphylococcus pyogenes albus I. from the Lister Institute collection in London, except that albus would not thicken condensed milk. It probably belongs to the same group that has been described by Conn, Esten, and Stocking (5) under the name Micrococcus lactis albidus. The description given there of "variety B" seems to best describe this thickening organism.

Variety B. composed most of the cultures isolated from sweetened condensed milk. These cultures differed from those of variety A in that they did not reduce nitrates to nitrites. They also failed to ferment lactose. In some instances they showed yellow pigment, and produced no change in litmus milk. This organism corresponds very closely to the Micrococcus lactis albidus referred to as "variety C" by Conn, Esten, Stocking (5).

It is evident that many organisms of common occurrence closely resemble these thickening organisms. They can only be distinguished from the thickeners by the thickening test. While they can be found in many places, as already shown, they are found in greatest numbers in sweetened condensed milk as found on the market. Results indicate that they can also be isolated quite readily from the air.

The cause of the thickening, while not studied from the chemical standpoint, can be explained to some extent. It was found that some of the organisms studied produced a high acidity in condensed milk, but did not thicken it. This would indicate that the production of acid alone is not responsible for the thickening. It was also noted that some of the organisms that were unable to ferment either sucrose or lactose produced thickening, and that they produced a rennet curd in litmus milk.

A summary of all of these observations would seem to indicate that the thickening process is caused by a renninlike enzyme produced by the organism.

Organisms that were able to thicken condensed milk were not found to be common in fluid milk.

The thermal death point of the organism being 63°C. in condensed milk, as reported by Greig-Smith (1), and 64°C. in this work, it is evident that, if it existed in the fluid milk, it would not survive the condensing process.

The bacterial content, determined by the plate method, of sweetened condensed milk shortly after its manufacture is a good indication of the sanitary conditions of the plant in which it was made. It is also an index of the number of thickening organisms one should expect to find. This being true, a sample of milk produced under good sanitary conditions, and therefore having a low bacteria count would be less likely to be subject to this type of spoilage.

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# THE INFLUENCE OF SOME BACTERIAL ENZYMES ON THE HEAT COAGULATION OF MILK*

WILLIAM C. FRAZIER

*Department of Agricultural Bacteriology, University of Wisconsin, Madison, Wisconsin*

## THE HEAT COAGULATION OF MILK

It is well known that milk in which much acid formation by bacteria has taken place will curdle when the milk is heated to boiling. Some preliminary experiments had indicated that not only acid formation by bacteria, but also the production of a rennin-like enzyme might influence the coagulation of milk by heat. Since this heat coagulability is important in the evaporated milk industry it was deemed worthy of further investigation.

Most samples of fresh milk can be heated at a temperature of 140°C. or more for a time without permanent coagulation taking place. Sometimes, however, samples of freshly drawn milk will coagulate on heating to the temperature of 115° to 120°C. at which evaporated milk is sterilized. Sommer and Hart (27) have shown that this coagulability of fresh milk is due to the lack of the proper balance of salts in the milk. An excess of calcium in the milk or an excess of phosphates or citrates over the normal balance of salts makes this milk normal.

While the heat coagulatility of a freshly drawn milk may be due to the relation of the salts in the milk, in an older milk bacterial growth may be an important factor. Hart and Sommer (17) have shown that the original potential acidity of the milk does not determine its heat coagulability, nor does the

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* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station. Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Received for publication September 15, 1924.

original hydrogen-ion concentration. Acidity increased by the addition of acid or its formation by microörganisms does, however, make this milk more easily coagulble.

Of the bacteria that curdle milk two groups might be made: One which brings about the curdling by means of acid alone, and the other which produces a rennin-like enzyme which takes part in the coagulation. To the first group belong most of the more common milk flora, the Streptococcus lactis group, the Coli-aerogenes group, the Bulgaricus group, and a few less important organisms. The rennin formers that occur in milk belong chiefly to the proteolytic sporeforming rods, mostly of the potato bacillus group; although some of the udder cocci produce rennin.

Experiments by Fay (10) and by Rogers (23) had indicated that the rennin produced by bacterial growth might have an effect on the coagulation of milk by heat.

## BACTERIAL RENNIN

Rennin is the active principle which causes the clotting or coagulation of milk casein, and is a term applied to the enzyme causing the coagulation, whatever its source may be. It was originally thought that only extracts from the stomachs or other animal organs contained rennin, but a multitude of workers have, during the past half century or so, showed the presence of rennin-like substances in simpler animals and in plants, molds, and bacteria.

That these rennins from different sources are not identical is indicated by their different physical and chemical properties. As the animal rennins differ, so do the rennin-like substances from plants and microorganisms differ among themselves from the animal rennins.

The sweet curdling of milk by bacteria has been noted by a number of different workers: Heubner (21), Duclaux (7), Warrington (30), Gorini (12) (13); Conn (6), and others. Of the organisms studied by them only those of the potato bacillus group and closely allied organisms occur commonly in milk.

Although they are generally not of much importance in its decomposition, at times they bring about sweet curdling in the absence of acid formers.

## THEORIES OF RENNIN ACTION

The coagulation or clotting of milk casein can be brought about by three general methods: (1) by action of an acid, (2) by action of a curdling enzyme, commonly called rennin, which may be of animal, plant, or bacterial origin, (3) by the action of heat.

In the coagulation of the casein by means of an acid like lactic acid the free protein is liberated from the original calcium caseinate with the formation of calcium lactate (61). The soluble calcium salt thus formed apparently causes the agglomeration of the small particles of colloidal protein and the subsequent precipitation.

The coagulation by rennin, however, does not find such a simple explanation. Space will not permit a discussion of the various theories of rennin action as advanced by: Hammarsten (15), Bosworth (5), Schryver (25) (26), Duclaux (8), Mellanby (19), Bang (3), and Alexander (1). All of the theories apparently indicate a belief that the rennin is not in itself a clotting enzyme, but acts on the original caseinogen molecule and modifies it in such a way that ordinary salt precipitation takes place. This would apply to rennin or rennin-like enzymes from any source, and would help explain the presence of these enzymes in living tissue where there is apparently no use for a curdling enzyme. To quote Falk (9) the action of the enzyme must be considered as a purely chemical catalytic action. In the case of casein precipitation by rennins, we are merely studying substances which place the milk in such a condition that the casein is insoluble in the presence of the existing combination of substances in solution.

## ISOLATION AND PURIFICATION OF RENNIN

The purification of an enzyme is always a matter of difficulty because there is generally a mixture of enzymes present, and because the enzymes resemble very much some of the organic compounds that always accompany them. So far as is known, no enzyme has yet been obtained in a very pure state. And when the method of action and the conditions necessary for the action of an enzyme are considered, it is doubtful whether it would be an advantage to have an enzyme in an absolutely pure state. It seems probable that the very process of bringing this organic catalyst down to a chemically pure state would destroy or modify the enzymatic action that is desired.

The argument about the identity of rennin and pepsin, however, has led to numerous attempts to separate and purify rennin and pepsin from commercial calves' rennet, and these methods have been extended to the study of rennin-like substances from plants and microörganisms. Hammarsten (15) (16) has published a number of different methods of separation. General methods of purification of enzymes include precipitation of the enzyme by alcohol, acetone, ammonium sulphate, lead acetate, or magnesium sulphate, accompanied by filtration or dialysis or both. The extraction of enzymes from yeast cells is accomplished by ether or chloroform and heat (21). Enzymes have also been purified by selective adsorption on aluminum hydroxide, Fuller's earth, finely divided carbon, etc., although always a great loss of enzymes has resulted. Reynolds (22) has described a method for the concentration and purification of enzymes by ultrafiltration through collodion membranes. While the above methods are fairly successful in partial purification of rennin from animals' stomachs, they do not seem to be as successful in the purification of bacterial enzymes from milk.

Conn (6) was the first worker to report on the isolation of bacterial rennin from cultures. He first grew the rennin-forming organism in milk, and when it had curdled filtered out the curd by means of a procelain filter. The enzyme was precipitated out with alcohol and redissolved in water. He obtained small amounts of rennin in this way. He then effected a partial separation of the rennin from the pepsin by precipitating the rennin with sodium chloride. He found that the porcelain filter held back much of the enzyme and that for that reason only a weak enzyme could be obtained.

Hata (18) filtered the curd from sweet curdled milk, evaporated the filtrate under reduced pressure and precipitated the enzyme with alcohol. The precipitate was treated with an excess of absolute alcohol and dissolved in water, and the enzyme was precipitated with ammonium sulphate. He obtained a mixture of pepsin and rennin.

## EXPERIMENTAL

### *Influence of milk cultures of bacteria on heat coagulation*

It was with the belief that the rennin produced by bacteria might have a considerable influence upon the heat coagulation of milk that the following work was first undertaken. Since Fay's work (10) was the basis for this belief it was thought advisable to check up his work, to some extent, on the effect of acidity, of rennet curdled milk, and of both on the curdling of milk by heat. In agreement with Fay it was found that:

*a.* Small amounts of acid inhibit heat coagulation over a short range.

*b.* Larger amounts of acid hasten heat coagulation.

*c.* Small amounts of a culture of a sweet curdling organism inhibit heat coagulation over a short range.

*d.* In the presence of little acid the addition of portions of a culture of a sweet curdling organism lowers the acidity at which heat coagulation will take place.

*e.* In the presence of larger amounts of acid this action of the culture is obscured or eliminated.

### *Influence of purified enzymes on heat coagulation*

When this part of the work was first begun it was with the hope that bacterial rennin could be isolated and separated from the other enzymes that might be present, especially the proteolytic ones. This, however, was not successfully accomplished, and all of the extracts of purified enzymes used below contained not only the rennin, but also proteolytic and possibly other enzymes. This was not deemed a great disadvantage, for in a general study of the effect of the sweet curdling organisms upon heat coagulation, the rennin enzyme would not be acting alone but in combination with the other enzymes of the organism.

The comparative strength of the enzyme extracts, isolated as described below, was first measured by Morgenroth's method (20) and later by modifications of this method. In Morgenroth's method a constant amount of enzyme solution is added to varying amounts of fresh milk to which one per cent of chloroform has been added. The tubes are placed at a temperature of from 0° to 5°C. for twelve hours or so, and then placed in a 37°C. water bath. The dilution at which curdling takes place within three or four hours is observed, and this taken as a measure of the strength of the rennin present. By this method very small amounts of curdling enzyme can be detected. The first modification made was the use of skim milk from powdered milk instead of fresh milk, as they were found to be equally efficient, while the skim milk was more constant in composition.

Later it was found that there was enough enzyme present in the extracts to test directly at 37°C., without the overnight incubation at 0°C. With the small tubes used it was found that the 37°C. incubator was as satisfactory as the 37°C. water bath. Observations were made at intervals of an hour up to the fourth or fifth hour. In some of the later work 0.6 per cent phenol was used as an antiseptic in place of 1 per cent chloroform. The phenol was not quite as satisfactory as the chloroform.

Several of the purified bacterial enzymes were used in the comparison of milks for use in the strength tests. Fresh milk-powder skim milk, sterilized (autoclaved) milk powder skim milk, pasteurized skim milk, and fresh whole milk were compared. Fresh milk-powder skim milk and fresh whole milk were found to be equally satisfactory for use with the bacterial rennin. Pasteurized skim milk was not as good, but could be used. The autoclaved milk powder milk was worthless. For use with chymosin or calves' rennet the fresh whole milk was superior to the other kinds.

The first attempts at the isolation and purification of bacterial rennin were made with the use of Conn's original method (6). In agreement with Conn, it was found that when the milk culture of the organism was filtered through Chamberland or Berkefeld filters that most of the enzymes were lost by absorption.

The next method attempted was similar to that used by Gerber (11) in the isolation of rennin from plant tissues. The milk culture of the sweet curdling organism was filtered through filter paper to remove the curdled casein. The filtrate was saturated, with ammonium sulphate. The coagulum, generally a scum, was skimmed off or filtered out and redissolved in a 5 per cent solution of NaCl. The solution was saturated again with ammonium sulphate. The coagulum was removed and placed in a parchment sac where it was dialyzed in running distilled water for several days. The resulting solution or suspension was used as freshly as possible and kept in the ice box until used. This method was fairly successful. Enzyme solutions were prepared which themselves were one-fourth to one-fifth of the original volume of milk culture, and which coagulated milk in dilutions as high as 1:50 or 1:100. If concentrated enzymes had been desired these solutions could have been evaporated under partial vacuum.

Collodion membranes were tried in place of parchment membranes but were not as good. This is explained by Bechhold (4) who found that rennin is absorbed in great amounts by collodion filters. It was also noted by Vandervelde (28) that a cellulose membrane was satisfactory for dialysis of a rennin containing solution.

The casein was removed from the original milk cultures with difficulty, particularly when an old culture was used in which considerable digestion had taken place. Attempts were made to remove this casein by various methods without the removal or destruction of the enzymes. The addition of acid was first tried. It was found that a very limited amount of acid could be used without much harm to the enzyme. Acetic and lactic acids were used, and if not over 0.5 per cent acid was added the enzyme was weakened but little. When the addition of acid was combined with heat, however, still less acid could be added.

The use of heat alone up to 50° to 55°C. gave a stronger enzyme solution than without heat. A temperature of 60°C. weakened the enzyme perceptibly. When heat and acid were combined very little acid could be used, for 0.25 per cent of acetic acid gave a weaker enzyme.

Lead acetate and calcium acetate were combined with heat with weakened enzymes as a result. The use of ethyl sulphuric acid was unsuccessful. Avery and Cullen (2) used bile in getting enzymes from the pneumococcus. This was not successful with the rennin enzymes of the sweet curdlers.

Most of the sweet curdling organisms used had an optimum growth temperature of 37°C. The optimum temperature for the production of rennin, however, was at lower temperatures of from 20° to 28°C.

More rennin was always found in an old culture, and one in which considerable digestion had taken place. Little enzyme was ever obtained from a culture that had freshly sweet-curdled. The rennin could be isolated from cultures which never curdled the milk but only digested it. The same observation was made as that by Gorini (14) and by Conn (6) that some of the organisms, which sweet-curdle milk when first isolated, lose that property on cultivation and have increased proteolytic power.

In attempts at the isolation and purification of the rennin from some 70 bacterial cultures in milk the following procedure was finally adopted as the most successful. The sweet curdling organism was inoculated into sterile milk and the milk incubated at 25°C. for five to six days. This culture was heated at 50°C. for fifteen minutes. If the casein separated out well, no acid was added, otherwise about 0.2 per cent glacial acetic acid was added upon removal of the milk from the 50°C. water bath. The culture was filtered through filter paper. The filtrate was saturated with crystalline ammonium sulphate; and the coagulum that formed was skimmed off and redissolved in water. This solution was saturated with ammonium sulphate, and the coagulum was skimmed off and placed in a parchment thimble (Schleicher and Schull). Dialysis was carried on for two days in running distilled water. If quantitative comparisons of the enzymes were to be made, measured amounts of culture were used and the final enzyme solutions were made up to definite volume. The enzyme solutions were kept in the ice box. Attempts to sterilize them by various methods failed.

Cultures of the organisms in nutrient broth were allowed to grow for several weeks to insure considerable autolysis of the cells. Little enzyme could be isolated from the cultures.

Direct dialysis of the milk serum gave a fairly strong enzyme solution, which was probably in a very impure state.

### *Heat coagulation tests with purified enzymes*

When the purified enzyme solution was added directly to milk in varying concentrations and the milk heated immediately at either 100° or 120°C. in no case was an effect on heat coagulation observed. Thirty trials were made with enzymes from ten different organisms with the same result in all experiments. All of these enzymes were strong enough so that one part of enzyme would curdle thirty parts of milk within three hours. When the enzyme has not been allowed to act on the milk, then, it has no effect upon heat coagulation. Indeed, a temperature of 50°C. for fifteen minutes would weaken the purified enzyme, while 60°C. would amost entirely destroy it. The same enzyme in the original milk culture was more heat resistant. This has been observed by other workers.

In order for the enzyme to have any effect upon heat coagulation, the enzyme must have been allowed to act upon the milk for some time. This is indicated by experiments of which the following is an example.

Two enzyme solutions $Q_2B$ and 10E, had been previously tested for strength. One part of $Q_2B$ would coagulate fifty parts of milk within four hours. Solution 10E would coagulate about thirty parts of milk in the same time. One part of either enzyme to four parts of milk would not coagulate at 100°C. for thirty minutes.

After incubation at 37°C. for different lengths of time, the varying dilutions of the enzymes in milk were removed from the incubator, the amount of coagulation noted and the tubes then heated at 100°C. for thirty minutes. The results are shown in table 1.

The results of the above experiment show that the heat apparently carries to completion the coagulation begun by the enzyme, if that action has gone far enough.

Similar results are obtained when the tubes are heated in the autoclave at 137°C. instead of 100°C.

The optimum temperature for the action of these rennin-like enzymes is about 40°C. or slightly higher. At 37°C., then, the temperature used in the above experiments, favorable conditions obtained for the action of the enzyme on the milk. If the enzyme is allowed to act on the milk at room temperature, however, there is still a marked influence on heat coagulation. When, for instance, the two enzymes used in the previous experiment were allowed to act on milk at room temperature for an hour, the only coagulation at that time was with 10E in a 1:5 dilution. When the tubes of milk were autoclaved, coagulation took place as follows:

TABLE 1

| ENZYME | TIME AT 37°C. | COAGULATION DILUTION | |
|---|---|---|---|
| | | After 37°C incubation | After thirty minutes more at 100°C. |
| | *minutes* | | |
| $Q_2B$ | 15 | 1:5 | 1:10 |
| 10E | 15 | Negative | 1:5 |
| $Q_2B$ | 30 | 1:10 | 1:15 |
| 10E | 30 | 1:5 | 1:10 |
| $Q_2B$ | 45 | 1:15 | 1:30 |
| 10E | 45 | 1:5 | 1:15 |

10E—1:5, 10, 15, 20, 30 = ++; 1:40 = negative
Pr —1:5, 10, 15 = ++; 1:20 = negative

Similar results were obtained with other enzyme solutions.

Even at the ice box temperature, 10°C., the enzyme has its effect, although no curdling is noted when the tubes are removed from the ice box. The following experiment with the same enzyme illustrates this point.

Dilutions of the two enzymes in milk were made; and the tubes were placed in the ice box at 10°C. for ten hours. At the end of this period there was no indication of curdling in any of the tubes. A set of tubes with each enzyme was immediately autoclaved at 20 pounds pressure for ten minutes. Coagulation took place as follows:

10E—1:5, 10, 15, 20, 30 = ++; 1:40, 50 = partial
Pr —1:5, 10 = ++; 1:15 = partial

As a check upon the ten hour action of the enzymes at 10°C., two sets of tubes were also placed at 37°C. and the rate and amount of coagulation noted. It will be observed that this procedure is really the same as Morgenroth's method for detecting very small amounts of rennin. As was expected the action was rapid and soon came to completion. Within fifteen minutes 10E had curdled at a 1:15 dilution, and Pr at 1:5. After an hour 10E had coagulated in a 1:40 dilution and Pr in 1:15.

The above experiments have shown that the bacterial rennin can act upon the milk in such a way as to favor heat coagulation, even though no outward sign of its action is to be observed. The rate of this enzyme action is dependent upon the temperature. As is the case with all enzyme actions and most chemical reactions, an increase in temperature increases the rate of action up to the optimum temperature. The rate of action of the bacterial rennins studied has been roughly proportional to the amounts of enzyme used.

Since the presence of calcium is necessary for coagulation by animal rennet, and since calcium also apparently plays a part in ordinary heat coagulation of milk, it was thought advisable to study the effect of calcium and citrates upon the action of bacterial rennin.

Comparable to the way in which the presence of calcium helps the action of animal rennin at ordinary temperatures (50) was the effect of calcium on the action of bacterial rennin. The addition of small amounts of calcium acetate (M/12) hastened the curdling perceptibly at room temperature and at 37°C. The addition of sodium citrate had the opposite effect, that is it inhibited enzyme action. These results would indicate that the action of bacterial rennin might not only be like the action of animal rennin, but might also be similar to that of the coagulation by heat due to a lack of the proper balance of milk salts. If this were true then the enzyme action should have an effect that would be additive to the effect of an excess of calcium in the milk salts. Experiments indicate that this is true.

In table 2 is shown the effect of the addition of calcium acetate and of sodium citrate in amounts proportional to the amount of enzyme added. M/12 calcium acetate was used, and M/4 sodium citrate; and one part of the salt solution was added for each part of enzyme. Thus a dilution of 1:30 would mean one part of calcium acetate solution to 29 parts of milk solution and also one part of enzyme to 29 parts of total solution. The salts and enzymes were added to varying amounts of milk and the tubes allowed to stand for an hour at room temperature. The amount of curdling, if any, was observed at this time; and then the tubes were heated in the autoclave at 125°C. for ten minutes.

TABLE 2

*Effect of calcium acetate or sodium citrate plus enzyme upon heat coagulation*

| SERIES NUMBER | ENZYME | SALT ADDED | DILUTIONS AT WHICH COAGULATION TOOK PLACE | |
|---|---|---|---|---|
| | | | At 22°C. for one hour | At 125°C. for ten minutes |
| I | 10E | | 1:5 | 1:30 |
| II | Pr | | Negative | 1:50 |
| III | 10E | Ca acetate | 1:15 | 1:40 |
| IV | 10E | Na citrate | Negative | 1:15 |
| V | Pr | Ca acetate | 1:10 | 1:30 |
| VI | Pr | Na citrate | Negative | 1:10 |
| VII | | Ca acetate | Negative | 1:15 |
| VIII | | Na citrate | Negative | Negative |
| Controls | | | Negative | Negative |

It will be noted in table 2 that the effect of the addition of calcium acetate on the action of the enzymes at room temperature is also shown, for coagulation has taken place at higher dilutions after one hour where the calcium salt was added. The important thing to be noted in the table, however, is how the effect of enzyme plus calcium acetate is practically an addition of the separate effects. On the other hand, the addition of sodium citrate had an inhibitive effect upon the heat coagulation.

The additive effect of the enzyme action and the action of calcium is again shown in table 3. In this experiment the amount of calcium acetate in the milk was constant, i.e., it was added to the whole lot of milk, while only the enzyme was varied in concentration. The amount of calcium acetate added was below the amount which would cause heat coagulation. If, now, heat coagulation took place at dilutions greater than those at which the enzyme action alone was effective, then it could be concluded that the action of enzyme plus calcium was additive. Heat coagulation was tested in the autoclave, where the tubes were heated at 20 pounds pressure for ten minutes.

As is shown in table 3, heat coagulation took place at twice as great a dilution when calcium acetate was present as when the enzyme was alone, even when the amount of calcium acetate present was not enough to cause heat coagulation.

A series of experiments was run to show that the influence of an excess of calcium was effective whether the enzyme had been acting at lower temperatures or at higher ones. Enzyme solutions from four different organisms were used. Two of the solutions, 10Ea and Pra. were comparatively strong and would coagulate milk in dilutions of 1:40 and 1:30, respectively, after two hours at 37°C. The other two enzyme solutions, $Q_3$a and BSRa, were weaker and coagulated milk in dilutions of 1:15 and 1:10 respectively.

TABLE 3

*Additive effect of calcium acetate and enzyme (at 125°C)*

| SERIES | ENZYME | DILUTIONS AT WHICH COAGULATION TOOK PLACE | |
|---|---|---|---|
| | | Without calcium acetate | With calcium acetate |
| I | 10E | 1:20 | 1:50(plus) |
| II | Pr | 1:20 | 1:50 |
| III | BSR | 1:10 | 1:20 |
| VI | $Q_3$ | 1:10 | 1:20 |
| Controls | | Negative | Negative |

A series of dilutions of the enzyme solution was made in milk-powder milk. A second similar series was treated with calcium acetate solution so that there was added one part of M/12 calcium acetate to each part of enzyme solution. In a third series the enzyme was added, the milk was treated with calcium acetate solution, so that the strength of the added calcium acetate was M/240. This was not enough to cause coagulation by heat.

The three series were incubated at the temperature desired and then tested for heat coagulation.

In table 4 is shown how calcium acetate plus enzyme action affect the heat coagulability of the milk, when incubation has been at ice box temperature (11°C.) for ten hours.

It will be noted in table 4 that in all cases the effect of the enzyme and the calcium acetate is additive. The expression of the strength of added calcium acetate at the heat coagulation dilution does not give an absolute measurement of the amount of effective calcium in all cases. With enzyme 10Ea, for instance, there seems to be a wide variation in the amount of added calcium acetate at the point of heat coagulation. The dilutions were made, however, only through 1:50; and coagulation would probably have proceeded farther. This would also hold for the third treatment with Pra. It will be noted, however, that when only calcium acetate is added to the milk that the presence of

TABLE 4

*Influence of calcium acetate and enzymes on heat coagulation*

Incubation at 10° for ten hours

| ENZYME | TREATMENT WITH Ca ACETATE | DILUTION AT WHICH COAGULATION TOOK PLACE | | STRENGTH OF Ca ACETATE AT HEAT COAGULATION DILUTION |
|---|---|---|---|---|
| | | At 10°C. for ten hours | At 120°C. for ten minutes | |
| 10Ea | a* | Negative | 1:30 | |
| 10Ea | b | 1:10 | 1:50 | M/600 |
| 10Ea | c | Negative | 1:50 | M/240 |
| $Q_3a$ | a | Negative | 1:10 | |
| $Q_3a$ | b | Negative | 1:15 | M/180 |
| $Q_3a$ | c | Negative | 1:20 | M/240 |
| Pra | a | Negative | 1:10 | |
| Pra | b | 1:10 | 1:30 | M/360 |
| Pra | c | 1:5 | 1:50 | M/240 |
| BSRa | a | Negative | 1:5 | |
| BSRa | b | Negative | 1:15 | M/180 |
| BSRa | c | Negative | 1:15 | M/240 |
| Control | Like b | Negative | 1:5 | M/60 |
| Controls | | Negative | Negative | |

* Treatment "a" = no calcium acetate added. Treatment "b" = one part of M/12 calcium acetate to one part of enzyme. Treatment "c" = strength of calcium acetate is M/240 in all dilutions of enzyme.

M/60 calcium acetate is necessary to bring about heat coagulation while in the presence of enzyme 10Ea heat coagulation took place with M/600 calcium acetate present.

When incubation took place at room temperature or at 37°C. the results were similar to those at ice box temperature.

The effect of enzyme and of calcium acetate is similar, then, whether the temperature of incubation is high or low. At higher temperatures the action of the enzyme is more rapid and would come to completion sooner than at lower temperatures. The results of this work has indicated that a definite quantity and strength of enzyme can accomplish only a certain amount toward making the milk heat coagulable. Even though the time of enzyme action may not have been sufficient to bring about coagulation at the temperature of incubation, the enzyme has acted upon the milk in such a way that the coagulation by heat will take place at as high a dilution as that at which coagulation would have taken place at the optimum temperature for enzyme action. In all of the above experiments controls kept at 37°C. have borne out this statement. The added calcium acetate has had a double effect on heat coagulation. It has not only per se made the milk more heat coagulable, but it has made the enzyme more effective.

As a matter of comparison the effect of chymosin or calves' rennet on heat coagulation was studied. The chymosin seems to act exactly like the bacterial rennin. As is well known, soluble calcium aids the action of the chymosin. When the chymosin had been allowed to act upon the milk for a short time, heat coagulation took place at that dilution at which coagulation at 37°C. would have taken place if the reaction had been allowed to go to completion.

It has been shown that the presence of soluble calcium in small amounts helps or hastens the action of bacterial rennin. That the presence of small amounts of acid would produce a similar effect is to be expected, for the acid would also serve to form soluble calcium salts. Experiments with six different enzymes have shown that the addition of small amounts of lactic acid hastens the curdling at 37°C. by bacterial rennin and carries the curdling out to higher dilutions.

## STUDIES OF ORGANISMS USED

A detailed study of the more important organisms used in the above experiments was made, according to the methods endorsed by the Society of American Bacteriologists. Most of the organisms belong to the potato bacillus group, but although they answer to the general characteristics of that group, they will not fall into any of the species mentioned by Bergey. It was not deemed necessary to record in this paper complete descriptions of the organisms. All of these potato bacillus-like organisms were spore forming rods, motile, and gram positive. All formed chains, mostly short. With the exception of B. subtilis they were facultative toward oxygen with a tendency toward aerobic. All sweet curdled milk with subsequent digestion, more or less complete. Some lost their ability to sweet curdle milk after a period of cultivation in the laboratory. All varied among themselves in certain details, and all were isolated from milk which had sweet curdled.

In addition to these spore forming rods, three non-spore forming saprophytes were used. Enzymes from B. pyocyaneus, B. fluorescense liquefaciens, and B. prodigiosus were purified and studied.

## DISCUSSION

The practical application of the influence of bacterial rennin on heat coagulation of milk would, of course, be in the evaporated milk industry. The influence of acid upon heat coagulation has long been known. Hart and Sommer (58) have shown the effect of an excess or lack of calcium in the milk salts upon heat coagulation. The results of the above experimental work have indicated that bacterial rennin also has an effect upon heat coagulation. It has been noted that the cause of heat coagulation in all three cases is dependent on the calcium salts in the milk. In coagulation by means of acid the release of calcium as a salt of the acid probably plays an important part. The heat coagulation of freshly drawn milk is generally due to an excess of soluble calcium. And the heat coagulation of milk which has been exposed to the action of bacterial rennin has been shown to be connected with the amount of soluble calcium in the milk.

The action of any one of the three above factors may be sufficient to bring about heat coagulation of milk upon sterilization. But since their action is similar, the combined action of any two or all three of the factors would be still more effective.

The application of these results to the evaporated milk industry is evident. Night's milk is rarely delivered to a condensery before the following morning. If bacterial growth has taken place in the milk before cooling, or if it takes place during the night due to insufficient cooling, bacterial rennin may be produced and allowed to act on the milk during the night, even at low temperatures in a way similar to that in the Morgenroth method described above. The development of a slight amount of acid will aid in the action. Or if after preheating and evaporation, some of the spore-forming, rennin-producing rods start to grow and produce rennin, then there will be a long time for its action on the evaporated milk when the milk is cooled and held over night for canning and sterilization the next morning. These spore-forming rods would after the preheating and evaporation processes be freed of competition with the common acid forming organisms which do not form spores.

The thermophilic organisms which produce a rennin-like enzyme may have an opportunity to function. The temperature of the vacuum pan would be favorable to their growth; and there would be time for the action of their enzymes when the evaporated milk is held over night, as shown above; the low temperature of storage would still permit the action of the rennin to take place, so that coagulation would take place on sterilization in the can the following day.

Some of the organisms used in this work grow very rapidly at favorable temperatures; sweet curdling has resulted within eight to ten hours at times.

The production of rennin-like enzymes by bacteria which occur in milk, then, is a factor which should be considered in the coagulation of a milk on sterilization.

## SUMMARY

1. The results of the first part of the work where only milk cultures of the sweet curdling organisms were used can be summed up as follows:

*a.* Small amounts of acid inhibit heat coagulation over a small range.

*b.* Larger amounts of acid increase heat coagulation.

*c.* Small amounts of a milk culture of a sweet curdling organism inhibit heat coagulation over a small range.

*d.* Larger amounts of a milk culture of a sweet curdling organism hasten heat coagulation.

*e.* In the presence of little acid the addition of portions of a milk culture of a sweet curdling organism lowers the acidity at which heat coagulation will take place.

*f.* In the presence of larger amounts of acid this action of the culture of a sweet curdling organism is obscured or eliminated.

2. The results of the second part of the work when the rennin-like enzymes of certain bacteria were isolated and purified are as follows:

*a.* The rennin-like enzymes of sweet curdling organisms were isolated and purified to some extent by methods described.

*b.* Bacterial rennin is destroyed by a temperature of 60°C. for fifteen minutes.

*c.* The purified bacterial rennin has no effect upon heat coagulation when the milk is heated immediately upon addition of the rennin.

*d.* When the rennin has been allowed to act on the milk for a time, the heat coagulability of the milk is increased.

*e.* The rate of this action of the rennin is dependent on the concentration or strength of the enzyme present.

*f.* The rate of action of the rennin is dependent on the temperature at which the enzyme is allowed to act. A temperature of 40° to 43°C. is the optimum for most rennins. An increase in temperature up to the optimum temperature hastens rennin action. Action goes on at a slow rate at temperatures as low as 10°C.

*g*. The addition of acid in small amounts accelerates the rennin action.

*h*. The presence of soluble calcium salts in small amounts accelerates the rennin action.

*i*. The presence of citrates in small amounts retards the rennin action.

*j*. The effect of acid plus rennin upon heat coagulation is additive.

*k*. The effect of soluble calcium salts plus rennin upon heat coagulation is additive.

*l*. A definite amount of curdling enzyme can cause heat coagulability only in a limited amount of milk.

*m*. This limit of influence is the same whether the rennin is allowed to complete its action at its optimum temperature or autoclave temperatures are applied after the action is started.

*n*. Chymosin resembles the bacterial rennins in its influence upon the heat coagulation of milk.

*o*. Bacterial rennin may be an important factor in the coagulation of milk upon sterilization in the condenseries. The rennin may work in conjunction with acid in the milk or an excess of soluble calcium.

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# IMPROVING DAIRY CATTLE BY THE CONTINUOUS USE OF THE PROVED SIRE*

R. R. GRAVES

*Dairy Cattle Breeding Investigations, Bureau of Dairying, United States Department of Agriculture, Washington, D. C.*

Recently there have appeared in the press such statements as the following: "Breeding production to production does not tend to result in further increase of productive capacity. It more often ends disastrously. It is now recognized by a steadily increasing number of leading breeders that the scales and Babcock test, great as have been their contributions to dairy advancement, can not safely serve alone in directing breeding plans."

There can be no question that breeding production to production, in the sense that this method of mating has been commonly followed, has not always brought desirable results. Inheritance for producing capacity has been gauged by breeders by the individual production record of a cow. If a bull is the son of a cow with a high record and is mated to a cow with a high record, this is breeding production to production and it is thought by many that the progeny of the mating should be a high producing animal. Frequently the results of this type of mating have been disappointing and have led to such statements as the one quoted. The difficulty is, that in the interpretation of the results of such matings, consideration has not been given to the fact that the inheritance of all animals for any given character, such as producing capacity, is double in its nature—that is, it has a double origin. Each individual possesses an inheritance for each character received from each parent, though outwardly the individual may for some features or characters show great resemblance to one parent, and in other features or characters show more marked resemblance to the other parent. Nevertheless, the individual does possess and will transmit an inheritance for each feature or character received from each parent, though it may be visible or

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* Received for publication May 10, 1925.

invisible. Hence the statement that all individuals are double in their inheritance.

The cow that has a large record does not necessarily have an inheritance from both parents for high-producing capacity. She may be mixed, that is, heterozygous, in her inheritance for producing capacity. From one parent her inheritance for producing capacity may be for high production and from the other parent her inheritance for producing capacity may be for low production. If the factors determining a high-producing capacity are dominant over the factors determining low producing capacity, this cow may be a high-producing animal and yet she may transmit a low producing capacity to half her offspring. Our failure to breed cattle that will be pure for an inheritance for high production by breeding production to production is not the fault of our production records, nor can we say that our production records are not indicative, but rather that we have not applied correctly the knowledge given by the production records to our selection and breeding methods. We have failed to take into account the double nature of the inheritance of each individual for each character.

In a number of papers and talks in the past few years the writer has advanced the theory that the sire's hereditary makeup for producing capacity is more accurately indicated by the production records of a number of his daughters than is the hereditary makeup of the cow for producing capacity, by her individual production record.

Where all the daughters of a sire are uniformly excellent producers, it would seem to indicate that such a sire has in his germinal makeup only factors that will determine high-producing capacity and, therefore, that he is pure—using the term "pure" as a synonym for the genetic term "homozygous"—insofar as hereditary factors controlling high-producing capacity are concerned. We commonly refer to such a sire as being prepotent for high production. This prepotent sire for high production may or may not be prepotent in transmitting good type also.

If the sire gets some good-producing and some poor-producing daughters, it would seem to indicate that he is either heterozygous in his inheritance for producing capacity, and will therefore transmit to some of his offspring the inheritance that will determine high-producing capacity and to some the inheritance that will determine low-producing capacity or else that he is homozygous for recessive factors determining low production and that a part of his daughters may be good producers because of their having received from their dams dominant factors determining high-producing capacity. It is realized that there are all degrees of producing capacity but the terms "low" and "high" are here used to illustrate extremes. The real test to determine the hereditary makeup of both the sire and the dam is the breeding test—the inheritance that is actually passed along to a number of offspring. Because of the fact that it is possible to measure the inheritance of the producing capacity of a larger number of daughters that are by a single sire, and in a short space of time, than is possible for any considerable number of daughters from a single dam, it is possible to determine more accurately the hereditary makeup of the sire than of the dam.

Our theory is, therefore, that improvement in dairy cattle breeding will be brought about most rapidly by the discovery and use of these prepotent sires that are pure for the hereditary factors determining high production.

It may be questioned whether unrelated sires of the same breed possess the same factors or combinations of factors that enable them to be prepotent. This remains to be proved but the indications are that they do possess them. If they do, then by the use of proved sires of the same breed for six or seven generations, the resulting progeny will approach the condition of being homozygous for the factors determining high-producing capacity. The continuous use, for generation after generation, of sires of proven ability for transmitting high-producing capacity, in an effort to breed a pure line for high-producing capacity, is one of the breeding projects which the Bureau of Dairying has under way.

We have several calves at the Bureau of Dairying experimental farms that have three, four and five direct generations of proved sires. This statement should be qualified in two ways. In the first place it is not meant to imply that all the ancestral sires in four generations are proved. If the sire of the individual in question is definitely proved, no further attention need be paid to his ancestry. It is possible for this sire to be homozygous for the inheritance determining high production and yet for both of his parents to be heterozygous in their hereditary makeup for the factors controlling producing capacity. In Jersey cattle, for example, spotting is recessive to solid color. We may have two solid-colored parents that carry the recessive factor for spotting and from these parents we may get calves at different matings that are (1) homozygous for spotting and are therefore spotted; (2) homozygous for solid color and are therefore solid color; or (3) heterozygous in their inheritance for color and spotting, but are of solid color, possessing and transmitting, however, the inheritance for both solid color and spotting, as were the parents. Therefore, if either the sire or dam is once definitely proved to be homozygous for the character desired, there is nothing to be gained, insofar as that particular character is concerned, by consideration of the ancestry.

The second generation sire to be considered would be the sire of the dam, and the third generation sire to be considered would be the sire of the maternal granddam and so on. In the accompanying chart only the sires that need be considered, providing they are proved, are shown. If each of these sires be homozygous for factors determining high producing capacity and if each has the same combination of factors, the only opportunity for the individual in question to have received recessive factors must have come through the maternal great granddam, granddam and dam. So that in speaking of four direct generations of proved sires we are actually referring to only one sire in each ancestral generation.

The second qualification is in the statement that each of these sires is proved to be homozygous for the hereditary factors determining high-producing capacity. To determine that a sire is homozygous for factors determining high-producing capacity is not as simple as it may appear. If a bull is heterozygous in the factors determining producing capacity and is mated with cows that are also heterozygous, some three-fourths of the daughters would be good or high producers and one-fourth poor producers.

If a sire is homozygous for the recessive factors determining low producing capacity and is mated with cows that are homozygous for dominant factors determining high-producing capacity, all the daughters should be good producers; they will be heterozygous in their inheritance, however, and will transmit low production to half their offspring. If a sire is mated with good cows it is a difficult matter to determine his hereditary makeup. A very large number of daughters from such matings would be required before his transmitting ability can be determined. It would seem that the best way to prove a sire would be to mate him with low-producing cows. Ordinarily, the low-producing cow would be homozygous for recessive factors determining low production unless she had some organic trouble that prevented the expression or fulfillment of her inheritance for high production. In the present stage of our breeding operations, when most cows are probably heterozygous for their inheritance governing producing capacity, a bull's hereditary makeup should probably not be estimated from the producing capacity of less than six daughters. As more cows become homozygous in their hereditary makeup for the dominant factors governing high-producing capacity, it will become more difficult to prove sires in such manner as to be certain of their being homozygous for dominant factors determining high producing capacity. Another factor that adds to the difficulty in determining the hereditary makeup of an advanced register or register of merit sire is the fact that breeders do not test the poor daughters of a sire due perhaps to not wanting the unfavorable publicity that such a record would bring, and due also to the high requirements for advanced registry and register of merit. As our testing of purebreds is handled to-day a sire that is homozygous for recessive factors determining low production, if mated to a few cows that are homozygous for dominant factors determining high production, and if only such daughters as are from these homozygous cows are tested, may acquire a reputation for being a great sire, whereas if the records of his poor daughters from poor cows were published it would give a more accurate indication of his real hereditary makeup. Certainly, breeding animals could be more intelligently selected if all records, whether good or poor, were published.

If the theory is correct that the use of proved sires for generation after generation will produce a strain that will be homozygous for dominant factors determining high production, then this same theory offers a method for the selection of bull calves that will prove to be prepotent sires.

So far as we know no attempt has been made to determine how many factors are concerned in the inheritance governing producing capacity. Perhaps the stimulation to secrete milk may be a simple inheritance. The good dairy cow may vary from the poor-producing cow only in that she has this greater stimulation to secrete milk. Does the dairy cow have a different capacity for the assimilation, digestion and conversion of large amounts of nutrients, in the form of grains and roughage, into dairy products than the beef animal that produces a limited amount of milk? Or is it all in this one difference, the stimulation to convert nutrients into milk, or the stimulation to convert nutrients into meat? (Certainly the cow will not be a greater producer than is permitted by the strength of her weakest link in the chain of essential functions of the organs entering into the scheme of digestion, circulation and secretion.) In the herd of the Oregon Agricultural College several years ago were four full sisters, daughters of a noted sire. Three of the daughters made good records, but the fourth daughter seemed incapable of making one. On the death of this daughter it was found that she had a leaky heart valve. Perhaps this daughter had the same inheritance for producing capacity as did her three full sisters but because of this organic defect she could not utilize this inheritance to the fullest extent.

Since the number of factors entering into the determination of producing capacity is not known, no accurate prediction can be made as to the probability of securing an animal that is homozygous for the factors determining high-producing capacity, as a result of three or four generations of proved sire matings. If we assume that the stimulation to secrete milk is the variable characteristic that causes the difference in producing ability between the high-producing and the low-producing cow and if we further assume that practically all cows have the digestive capacity to consume, digest and assimilate large amounts of nutrients for the production of large amounts of milk and butterfat, and have the constitution that will enable them to withstand the consequent tax on their physiological makeup, we could, perhaps, predict the results of several generations of proved sire matings. Let us assume, for purpose of illustration, that producing capacity in dairy cows is controlled by the intensity of the stimulation for secretion and that this is controlled by a single pair of factors. Refer to the chart showing three generations of proved sire matings and let us see what the chances are for this heifer calf being homozygous for a high-producing capacity. If all three of the sires whose pictures appear on this chart are homozygous for the factors determining a high-producing capacity, any recessive factors for low production that might be a part of the hereditary makeup of this heifer calf must come from the maternal great-grandam, Katrine of Hillside. The picture of Katrine of Hillside does not appear on this chart, but she was mated to Hillside Torono and produced the cow 403. Katrine of Hillside has no record and we know nothing about her. Let us first assume that Katrine of Hillside is homozygous for recessive factors determining low production. The open circle will indicate the recessive factor for low production, the solid circle the dominant factor for high production. The pedigree follows:

| | | | |
|---|---|---|---|
| | Interested Veda's Prince<br>●● | Tiddledywink's Raleigh<br>●● | Heifer #639<br>●●, ●●, ●●,<br>or ●O |
| Hillside Torono<br>●● | Cow #403<br>●O | Cow #457<br>●● or ●O | |
| Katrine of Hillside<br>OO | | | |

Thus we see that cow 403 is heterozygous for the factors determining producing capacity; that the chances are equal for cow 457 being either homozygous for the factors determining high produc-

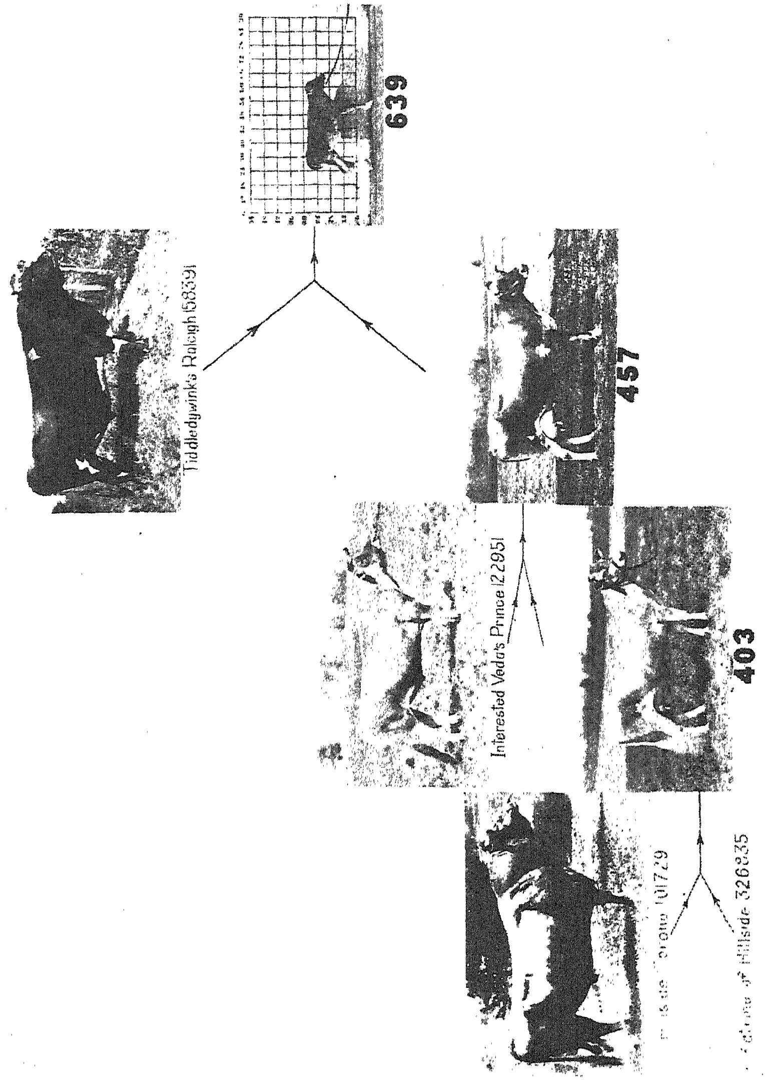
Tiddledywink's Raleigh 158391
639
457
Interested Veda's Prince 122951
403
101729
of Hillside 326835

FIG. 1

tion or heterozygous for factors determining producing capacity; that the chances are 3 out of 4 for heifer 639 to be homozygous and 1 out of 4 for her to be heterozygous.

Let us assume that Katrine of Hillside is heterozygous for factors determining producing capacity and see how this may affect the chances of heifer 639 for being homozygous for factors determining high producing capacity.

Hillside Torono
●●
Katrine of Hillside
●O

Interested Veda's Prince
●●
Cow #403
●● or ●O

Tiddledywink's Raleigh
●●
Cow #457
●●, ●●, ●●,
or ●O

Heifer #639
●●, ●●, ●●,
●●, ●●, ●●
●●, or ●O

Thus it will be seen if Katrine of Hillside is heterozygous for the factors determining producing capacity, and if these three sires are all homozygous for the factors determining high producing capacity, heifer no. 639 has 7 chances out of 8 of being homozygous for factors determining high producing capacity. These calculations were made solely on the basis of the inheritance for producing capacity being a simple inheritance controlled by one set of factors. We know that such may probably not be the case, but we do not know how many factors are involved or have a bearing on the ultimate producing capacity. However, when a sire is transmitting uniformly high-producing capacity to his daughters, no matter how many factors are concerned, then we can safely assume that he must be homozygous for the dominant factors concerned, else his daughters would not be uniformly high producers. Therefore, we believe that this serves as an illustration of a method for breeding for a pure line in high production and also as a means of selecting young bulls.

There is one other phase of inheritance in milk and butterfat-producing capacity that should be briefly discussed. Wherever there has been a cross between two distinct breeds of dairy cattle that have a considerable difference between their milk flow and range of butterfat percentage, such as the Holstein-Friesian cross on Guernseys or Jerseys, it has been observed that the resulting progeny have a milk flow and a percentage fat that is intermediate between the two parents. In these crosses the inbred $F_2$ generation has not shown a segregation of characters such as might be expected. This, together with a common belief of live stock breeders that a sire and a dam have a certain level of producing capacity and that the progeny will have a producing capacity that will represent an average of the parental levels, has led to a belief that the inheritance for milk and butterfat-producing capacity is a blending inheritance.

In the crosses between two distinct breeds there are probably so many independently inherited factors having a bearing on the milk flow and percentage fat in each parent that it is almost impossible to bring about in any limited number of animals a segregation of factors that will result in the exact reappearance of either of the parental characteristics, or of the desired combinations of the characteristics of the two parents. According to Coulter,[1] if two races of corn were crossed in which one parent had large grains, but those were few in number, and the other parent had small grains but many in number, the $F_1$ progeny would have grains intermediate in size and number. Now if the $F_1$ plants were inbred in the hope of obtaining a combination of the two extremes of large and also numerous grains, and if there were five factor pairs that influenced these quantitative characters in each case, in order to secure the desired pure type from this cross of two parents, each having five pairs of factors influencing their respective desired character, it is estimated that 100 acres of corn would have to be grown in order to have an even chance of securing one plant having the desired combination.

While in crosses between breeds having markedly different hereditary makeups as to such characters as milk flow and percentage fat, the following generations do seem to possess an intermediate milk flow and percentage fat, yet there is not the same evidence in crosses within a breed. No average or intermediate production such as is found in the crosses between distinct breeds will be found in the production records of the daughters of a single sire and their dams. The production records of the purebred daughters of an individual sire will be quite variable and cover a considerable range. A high record daughter may come from a low-producing dam and a low-producing daughter may come from a high-producing dam, or from two dams of approximately the same producing capacity may come daughters with records that vary widely. We have explained in this paper how that may very well come about through the dominant factors for high production and recessive factors for low production and the double nature of each individual in its hereditary makeup. In this discussion we have used the expression high and low producing capacity as illustrating extremes. There is need of further explanation, however, concerning the reason why some prepotent sires consistently raise the producing level of daughters coming from dams having records of 500, 600 and 700 pounds butterfat. (See table 1, showing records of daughters of prepotent sire and their dams.) The most logical

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[1] Coulter, "Outline of Genetics."

TABLE 1

*An example of a prepotent sire*

| DAUGHTERS | | | DAMS | | | DIFFERENCE | | |
|---|---|---|---|---|---|---|---|---|
| Milk | Fat | | Milk | Fat | | Milk | Fat | |
| *pounds* | *per cent* | *pounds* | *pounds* | *per cent* | *pounds* | *pounds* | *per cent* | *pounds* |
| 14,191.8 | 5.09 | 724.7 | 8,219.7 | 5.85 | 482.3 | +5,971.9 | −0.76 | +242.4 |
| 12,869.4 | 5.35 | 690.3 | 8,503.1 | 4.63 | 385.3 | +4,366.3 | +0.72 | +305.0 |
| 11,153.6 | 4.81 | 537.2 | 7,728.9 | 5.09 | 393.2 | +3,424.7 | −0.28 | +146.0 |
| 11,190.8 | 5.79 | 635.6 | 9,079.8 | 5.83 | 521.4 | +2,111.0 | −0.04 | +114.2 |
| 9,522.9 | 4.85 | 462.2 | 6,979.8 | 5.72 | 389.3 | +2,543.1 | −0.87 | +72.9 |
| 9,133.7 | 5.59 | 498.7 | 8,918.4 | 4.59 | 410.4 | +215.3 | +1.00 | +83.3 |
| Ave. 11,343.7 | 5.25 | 591.5 | 8,238.3 | 5.29 | 430.3 | +3,105.4 | −0.04 | +161.2 |
| Percentage increase or decrease.................. | | | | | | +37.69% | −0.76% | +37.46% |

The records of the daughters of this sire appear in the columns on the left and at the right of each daughter's record is the record of the dam. This sire increased the milk producing capacity of each daughter. The per cent fat of the daughters was decreased in all but two cases, but the decrease in per cent fat was not sufficient to offset the increase in milk and consequently every daughter showed an increase yield of butterfat.

explanation would seem to be that of the cumulative or multiple factor hypothesis. The cumulative factor hypothesis explains variations in quantitative inheritance such as height, yield, number of rows of grain on ear of corn, length of ear, etc. The theory is that quantitative characters are produced by cumulative factors—a factor that when added to another similar factor, affects the degree of development of that character. The theory is illustrated by Correns[2] experiment in crossing red and white strains of Mirabilis. Instead of the $F_1$'s being red as was expected, they were pink; intermediate between red and white. When these pink—$F_1$'s—were interbred a ratio of 1 red : 2 pink, 1 white was secured, the explanation being that a single dose of the determiner for red gives pink, while when the $F_1$'s were interbred a double dose of the red determiner gave red. Similarly, varying degrees of redness in wheat were secured in experiments at the Swedish Experiment Station[3] in which wheat with red and white kernels were crossed, the degree of red in the crossbred kernels depending upon the number of doses of red received.

An interesting case[4] of an intermediate result in crossing two varying characters was secured by Phillips in crossing two breeds of ducks—Rouens and Mallards—of different sizes. The Rouens were more than twice as heavy as the Mallards. The $F_1$ birds were intermediate in weight between the Rouen and Mallard parents, and due to the number of independent factors influencing the weight the $F_2$ birds did not segregate, but were also intermediate in weight, though more variable than the $F_1$ birds. None of the $F_2$ birds were as heavy as the Rouens, nor as light as the Mallards.

Punnett and Bailey[5] crossed two breeds of fowls differing widely in weight. The weight of the $F_1$ birds was much closer to that of the larger parent breed than to that of the smaller parent breed. This was taken to mean that one or more of the factors influencing large size showed dominance. Extreme variants were scarce in the $F_2$ generation, due to the small number of birds raised, but the $F_3$ birds had a great variation in weight, covering the ranges of both parent breeds. Individuals representing the extremes of the $F_3$ variants were mated and broods secured which averaged larger than the large parent, and smaller than the small parent. The interpretation was that there were four independent factors having among themselves unequal influence on the total weight; that when none of these factors were present birds of the minimum size were secured. Recombinations which included all four factors produced a race larger than the large parent. Similarly, recombinations which included the four allelomorphs of these factors produced a race smaller than the smaller parent. It was also assumed that two of the four hypothetical factors exerted a greater influence on total size than the remaining two factors.

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[2] Correns, C., Die neuen Vererbungsgesetze, Berlin, 1912.

[3] Nilsson, Ehle, Bot. Notiser 1908.

[4] Jour. Exper. Zool., 12, 1912; ibid., 1914.

[5] Journal of Genetics, 4, 1914.

Coulter says that the results of such experiments are in accord with the Mendelian method of inheritance, only two assumptions being necessary: (1) that dominance is absent, two doses having twice the effect of one; (2) that the independent similar factors are cumulative in their operation.

. . . . In connection with the cumulative factor concept, a modifying statement should be made as to the mechanism involved. Heretofore it has been assumed that we are dealing with numerous, separately inherited factors, absolutely identical in their nature, cumulative in their effect. No doubt one might regard with suspicion such a seemingly artificial mechanism.

Probably it would be easier to believe if it were modified in the following manner. Instead of assuming that the numerous factors are identical in function, we may assume that each factor has its own peculiar function, but that the function plays a part, directly or indirectly, in developing the quantitative character in question. For example, suppose height is the character. One of the factors determines the development of numerous nodes; another increases the amount of chlorophyll; another determines the size and vigor of the root system; another brings early germination and a long growing season. Such factors, although not identical, will be cumulative in increasing the height of the plant. Of course, a single dose of one type of factor may not bring the same increase in height as would a single dose of one of the other types, and therefore the mathematics of the situation will be slightly modified.

Applying the cumulative factor theory to producing capacity of milk and butterfat, if we assume that the presence of eight pairs of cumulative factors was necessary for the complete expression of an 800 pounds butterfat producing capacity, an animal with only four pairs of cumulative factors would have a producing capacity varying about the 400 pounds butterfat mark. If a sire were found that was homozygous for the eight factors necessary for an 800 pounds butterfat capacity and he were mated with heterozygous cows having enough of these cumulative factors to permit a 400 pounds butterfat capacity, then if the animals resulting from this mating were inbred, animals would be secured that would have producing capacities ranging from 400 to 800 or more pounds butterfat. This would seem to be in accord with what actually does happen in breeding work.

The great problem that has to be faced by the man who would breed uniformly high producing cattle, is that the hereditary material controlling producing capacity with which he must work is not purified or standardized. In order to make definite progress he must know the purity of the material with which he is working. The producing capacity of an individual cow is not a sufficient guide as to the purity of her germinal plasm for producing capacity, but the producing capacity of a number of daughters of a sire, especially if they be out of dams of known low producing capacity does give us a measure of the purity of the germinal plasm of the sire in regard to this character. It would seem to make little difference whether the theory of complete dominance or the theory of cumulative factors be followed. It is still the exceptional prepotent sire that offers to us the most rapid means of purifying the inheritance of our dairy cattle for high producing capacity. Coulter says: "We now know that individual variations are not always mere fluctuations or responses, but may be due to varying doses of cumulative factors. A selection on this basis may very well result in a new race that breeds true; and a race that breeds true is De Vries' definition of a new species." And so by the use of proved prepotent sires for generation after generation we may finally breed dairy cattle that will breed true for a high milk and butterfat producing capacity.

# THE APPARENT DIGESTIBILITY OF LOW PROTEIN RATIONS BY DAIRY COWS*

A. E. PERKINS AND C. F. MONROE

*Ohio Agricultural Experiment Station, Wooster, Ohio*

Continuing an investigation previously reported (1) a metabolism experiment was conducted with four Holstein-Friesian cows. The continuation was planned primarily with regard to the metabolism of minerals, which phase of the work is reported elsewhere (2): To these articles the reader is referred for further information regarding the cows; also regarding the details of the experimental methods.

The behavior of these animals with respect to the digestibility of the ration seemed worthy of record and is here presented.

The present experiment continued for two periods of 18 days each, which were separated by an interval of 12 days. Each 18-day period was divided into three 6-day sub-periods for each of which separate samples of milk, excreta and refuse were prepared. As the figures for the separate sub-periods agreed well with each other, only the average figure enters into this discussion.

The ration used is shown in table 1. It had a nutritive ratio of approximately 1:11; and contained approximately 70 per cent as much true protein and 158 per cent as much net energy as called for by Armsby's Standard (3); and 76 per cent of the digestible crude protein and 134 per cent of the digestible carbohydrates and fat called for by Haecker's Standard (4).

Cows 146 and 154 had been used in our previous experiments and had been receiving low protein rations very similar to those employed in the present work for several months. Cows 163 and 203, on the other hand, were taken shortly before the beginning of the present experiment from the regular herd where they had received pasture in season and an abundant supply of protein. Each of the cows was producing approximately 30 pounds milk per day at this time.

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* Received for publication May 4, 1925.

TABLE 1

*Amount of feed supplied in daily ration; analysis and average coefficients of digestibility analysis stated in percentage*

| KIND OF FEED | AMOUNT FED | DRY MATTER | AVERAGE DIGESTIBILITY COEFFICIENT | CRUDE PROTEIN | AVERAGE DIGESTIBILITY COEFFICIENT | ETHER EXTRACT | AVERAGE DIGESTIBILITY COEFFICIENT | CRUDE FIBER | AVERAGE DIGESTIBILITY COEFFICIENT | ASH | NITROGEN-FREE EXTRACT | AVERAGE DIGESTIBILITY COEFFICIENT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *pounds* | | | | | | | | | | | |
| Wheat bran | 3.25 | 90.30 | 0.65 | 14.75 | 0.78 | 3.90 | 0.68 | 10.21 | 0.31 | 7.13 | 54.31 | 0.72 |
| Ground corn | 6.50 | 88.25 | 0.90 | 9.31 | 0.74 | 3.16 | 0.93 | 2.05 | 0.57 | 1.20 | 72.53 | 0.94 |
| Ground oats | 3.25 | 91.65 | 0.70 | 12.69 | 0.78 | 3.33 | 0.87 | 11.77 | 0.35 | 3.73 | 60.13 | 0.81 |
| Dry beet pulp | 9.80 | 90.30 | 0.75 | 8.81 | 0.52 | 0.40 | 0.90 | 19.16 | 0.83 | 2.85 | 59.08 | 0.83 |
| Cane molasses | 1.30 | 73.90 | 0.78 | 2.69 | 0.32 | | | | | 5.09 | 66.12 | 0.90 |
| Timothy hay I | 10.50 | 87.26 | 0.55 | 3.71 | 0.48 | 1.47 | 0.50 | 32.51 | 0.50 | 3.96 | 45.62 | 0.62 |
| Timothy hay II | 10.50 | 86.72 | 0.55 | 4.22 | 0.48 | 1.35 | 0.50 | 31.35 | 0.50 | 4.15 | 45.65 | 0.62 |
| Refuse | | 95.75 | | 3.27 | | 0.33 | | 46.10 | | 4.46 | 41.59 | |
| Total nutrients supplied | I | 30.622 | | 2.784 | | 0.633 | | 6.140 | | | 19.873 | |
| | II | 30.556* | | 2.827* | | 0.621* | | 6.018* | | | 19.876* | |
| Digestible nutrients | I | 21.472 | | 1.790 | | 0.483 | | 3.579 | | | 15.835 | |
| | II | 21.436* | | 1.811* | | 0.477* | | 3.518* | | | 15.837* | |
| Calculated digestibility, entire ration | I | 70 | | 64 | | 76 | | 58 | | | 80 | |
| | II | 70 | | 64 | | 77 | | 58 | | | 80 | |

* The supply of nutrients for cow 203 in period II was 95 per cent of these values.

The sole difference in the treatment of the cows during the two periods of the experiment was in the kind of water supplied. One of each pair of cows, as indicated above, received distilled water and the other well or tap water during period I. In period II the kind of water supplied each cow was reversed.

The rations were well consumed but there was a small amount of refuse consisting mostly of the coarse stems of weeds which occurred as an impurity in the hay. This refuse was carefully saved and analyzed. The total amount for the four cows during the 36 days was only 3 pounds of air-dry material. Although the results have been calculated taking full account of these values, they do not enter into the present report because they merely introduce another complication without materially affecting the outcome.

TABLE 2

*Constituents of average daily feces*

| COW | PERIOD | WEIGHT OF FECES | WEIGHT OF AIR-DRY MATTER | DRY MATTER | CRUDE PROTEIN | ETHER EXTRACT | CRUDE FIBER | NITROGEN-FREE EXTRACT |
|---|---|---|---|---|---|---|---|---|
| 146 | I | 82.99 | 12.098 | 11.495 | 1.301 | 0.386 | 3.189 | 5.061 |
| 146 | II | 81.47 | 12.166 | 11.575 | 1.334 | 0.390 | 3.313 | 5.522 |
| 154 | I | 69.95 | 11.307 | 10.722 | 1.263 | 0.412 | 3.464 | 5.435 |
| 154 | II | 75.53 | 11.988 | 11.365 | 1.338 | 0.392 | 3.363 | 5.556 |
| 163 | I | 71.83 | 10.626 | 10.072 | 1.273 | 0.376 | 2.964 | 4.667 |
| 163 | II | 83.26 | 11.562 | 11.001 | 1.337 | 0.420 | 3.148 | 5.206 |
| 203 | I | 89.13 | 12.349 | 11.725 | 1.391 | 0.429 | 3.289 | 5.705 |
| 203 | II | 92.94 | 12.354 | 11.655 | 1.311 | 0.386 | 3.287 | 5.693 |

The results are presented in a series of four tables. Table 1 deals with the amount and composition of the daily ration. Table 2 considers in like manner the amount and ingredients of the average daily feces. Table 3 shows the amount, also the per cent, of each ingredient of the ration apparently digested. As a part of table 3 the observed digestibilities are grouped with respect to the previous feeding of the cows; those from cows 146 and 154 whose feeding had not been changed being compared with those from cows 163 and 203, recently changed from a ration of widely different character. The observed differences

TABLE 3

*Material apparently digested; observed percentages of digestibility*

| COW | PERIOD | DRY MATTER | PER CENT DIGESTED | CRUDE PROTEIN | PER CENT DIGESTED | ETHER EXTRACT | PER CENT DIGESTED | CRUDE FIBER | PER CENT DIGESTED | NITROGEN-FREE EXTRACT | PER CENT DIGESTED |
|---|---|---|---|---|---|---|---|---|---|---|---|
| D 146 | I | 19.127 | 62.5 | 1.483 | 53.3 | 0.247 | 39.0 | 2.951 | 48.1 | 14.812 | 74.5 |
| T 146 | II | 18.941 | 62.0 | 1.493 | 52.8 | 0.231 | 37.2 | 2.705 | 44.9 | 14.354 | 72.2 |
| T 154 | I | 19.900 | 65.0 | 1.521 | 54.7 | 0.221 | 34.9 | 2.676 | 43.6 | 14.438 | 72.7 |
| D 154 | II | 19.145 | 62.6 | 1.489 | 52.7 | 0.229 | 36.9 | 2.655 | 44.1 | 14.320 | 72.0 |
| D 163 | I | 20.550 | 67.1 | 1.511 | 54.3 | 0.257 | 40.6 | 3.176 | 51.7 | 15.206 | 76.5 |
| T 163 | II | 19.515 | 63.9 | 1.490 | 52.7 | 0.201 | 32.4 | 2.870 | 47.7 | 14.670 | 73.8 |
| T 203 | I | 18.897 | 61.7 | 1.393 | 50.0 | 0.204 | 32.2 | 2.851 | 46.4 | 14.168 | 71.3 |
| D 203 | II | 17.373 | 60.0 | 1.374 | 51.2 | 0.235 | 34.6 | 2.731 | 42.5 | 14.183 | 69.9 |
| 146 and 154, average (continuous low protein cows)........ | | | 63.0 | | 53.4 | | 37.0 | | 45.2 | | 72.9 |
| 163 and 203, average, (regular herd cows).................. | | | 63.2 | | 52.1 | | 35.0 | | 47.1 | | 72.9 |
| Tap water average............ | | | 63.2 | | 52.6 | | 34.2 | | 45.7 | | 72.5 |
| Distilled water, average........ | | | 63.0 | | 52.9 | | 37.7 | | 46.6 | | 73.2 |

in digestibility are so small that they lack significance in view of the unavoidable errors attending such work.

The results are also grouped with reference to the kind of water with which the cows were supplied. It should be noted in this connection that practically the same amount of each kind of water was consumed. Here again, the observed digestibilities are close and show no significant difference which can be ascribed to the influence of the kind of water used.

In table 4, the observed digestibilities are arranged for comparison with those calculated by the use of average coefficients (14). Striking differences are at once evident, the observed digestibilities being lower in every case. In the third line the observed digestibilities are expressed as per cents of the calculated.

TABLE 4

*Comparison of digestibilities*

| | | DRY MATTER | CRUDE PROTEIN | ETHER EXTRACT | CRUDE FIBER | NITROGEN-FREE EXTRACT |
|---|---|---|---|---|---|---|
| 1 | Observed digestibility from table 3 | 63.1 | 52.8 | 36 | 46.1 | 72.9 |
| 2 | Calculated digestibility from table 1 | 70 | 64 | 76 | 58 | 80 |
| 3 | Relation of observed to calculated digestibility, per cent: | | | | | |
| | This experiment (1 ÷ 2) | 90 | 82 | 47 | 80 | 91 |
| 4 | Wide ration cows 1921–1922 | 94 | 88 | 70 | 87 | 96 |
| 5 | Narrow ration cows, 1921-1922 | 95 | 94 | 89 | 90 | 99 |
| 6 | Cow 154, 1922, experiment | 94 | 91 | 66 | 88 | 94 |
| 7 | Cow 154, present, 1923–1924 experiment | 91 | 84 | 47 | 76 | 90 |

In lines 4 and 5 are give like figures taken respectively from the cows receiving wide and those receiving narrow rations in our previous work (1). Lines 6 and 7 give like data for cow 154 for the 1922 and the present experiment, respectively. The percentages are smaller, or in other words, the discrepancies between calculated and observed digestibilities were larger for each ingredient of the ration in the present experiment.

In both our experiments the greatest depression of digestibility occurred with the ether extract. Ellett and Holdaway (5) have observed depressions of digestibility accompanying the use of a wide or high-energy ration, which in the average are much greater than any we have observed. In their case the ether extract was less affected than any of the other ingredients. Their observations were made on cows producing only small amounts of milk.

Ellett, Holdaway, and Harris (6) have conducted extensive digestion experiments on dairy cows. The results of individual experiments fluctuated considerably but the mean apparent digestibility of the entire basil ration was about 3 per cent below that calculated from average figures. The addition to the ration of 1 pound starch increased this discrepancy to five per cent. A similar addition of 1 pound Black Albumin to the basil ration brought the observed to a practical equality with the calculated digestibility, and the authors conclude from this evidence that the addition of protein to a basil ration increases the digestibility of same. This coincides in a general way with the older view as expressed by Armsby (7) and Kellner (13). For the narrow ration cows in our earlier experiment (1) (see also line 5 in table 4) the observed digestibility was considerably below the calculated, even though all the cows were receiving an excess of protein and the ration of one of them had a nutritive ratio of 1:2.

Eckles (8) also reported digestibilities considerably below the calculated for his cows during liberal feeding and production on a ration carrying an abundant supply of protein; though the same cows fed a reduced amount of the same ration when dry appeared to digest more than the calculated amount.

The "Black Albumin," probably a dried blood preparation, used by Ellett, Holdaway and Harris in these experiments belongs to that group of highly digestible animal protein feeds, such as milk in its various forms, meat meal, tankage, fish meal, etc., various members of which have been shown to possess superior nutritive qualities (9) and to affect marked increase in the digestibility of rations of which they form a part (10); and some of which in experiments where digestibility was not determined have been shown to be more efficient milk producers than the usual high protein feeds of vegetable origin (15) (16). Dried blood is not a common ingredient of dairy rations and in view of our own results and those of Eckles we are obliged to question whether a like increase in digestibility would have been obtained by the addition of a like amount of protein from the usual sources. Indeed the digestibilities observed by Ellett and Holdaway (5) on their extreme high protein rations, from vegetable sources only, were not on a par with the calculated values.

The depression of digestibility observed in the present experiment was considerably greater than that which occurred in the case of cow 154 in the 1922 work (1) although the nutritive ratio of the two rations was the same, 1:11, indicating that some other influence aside from the amount of protein was at work. The chief points of difference between the two rations was that in 1922, 4.5 pounds clover hay was fed while no clover was used in the later work. The 1923–1924 ration contained 1.3 pounds cane molasses and 3.25 pounds oats not found in that of 1922. The difference may likely have been due to the specific influence of one of these feeds. Suspicion at once points to the cane molasses as the feed responsible for this depression of apparent digestibility, for Lindsey and Smith (11) working with sheep observed regular and consistent depression in the digestibility of rations of hay, hay and corn meal, and hay and gluten feed when cane molasses in varying amounts was added. The depression, however, was not proportional to the amount of molasses fed. Williams (12) has very recently reported somewhat irregular depressions in the digestibility of dairy rations resulting from the rather liberal use of molasses. He considered the molasses as 100 per cent digestible, hence did not include the nutrients supplied by the molasses along with the other food in calculating the digestibilities of the total ration. Had these values for the molasses been included the depressions in digestibility for the entire ration would have been greater.

Kellner (13) reviewing the work of numerous European investigators regarding the depression of digestibility caused by the addition of carbohydrates such as, starch or sugar, suggests the following possible causes which would apply equally well in case of molasses.

The depression in the digestibility of protein is commonly attributed to the increase in nitrogen excretion via the intestines which always accompanies an increase in food, even of nitrogen-free food.

Other causes are suggested some of which may affect the protein as well as the other nutrients of the ration.

*a.* The following by the intestinal organisms of a kind of path of least resistance, by working on the easily fermentable material added rather than on the more resistant feed residues which they would otherwise attack.

*b.* The production of considerable amounts of lactic acid from the starch or sugar, lactic acid having an inhibitory effect on bacterial action.

Increased peristaltic action which does not allow sufficient time for the completion of the digestive process has also been suggested as a probable cause, although this could not be verified by Lindsey and Smith (11).

Our data do not afford any further explanation as to the immediate cause of the depression as observed in our experiment, except that the depression of protein digestibility in case of cow 154 could hardly have been due to the presence of additional carbohydrates, for the nutritive ratio was the same in each experiment.

The data do, however, permit of an interesting comparison with respect to the ultimate effect of such apparent depressions in digestibility on the productive value of the ration.

In the case of cow 154 the ration used during the 1922 metabolism experiment as well as that used in 1923–1924 was representative of that fed during the entire course of the respective lactation periods. In the 1922 period a total of 6567 pounds digestible nutrients, figured from average coefficients, was consumed and a production of 9646 pounds milk containing 342 pounds fat recorded in 363 days, a production of 1.47 pounds milk containing 0.052 pound fat for each pound of digestible nutrients consumed.

In the succeeding lactation period on the ration having a much lower apparent digestibility as shown in table 4 she consumed

7818 pounds digestible nutrients figured by average coefficients and produced 11,793 pounds milk containing 364 pounds fat in 366 days, a production of 1.51 pounds milk containing 0.047 pound butterfat for each pound of digestible nutrients consumed. Values very close indeed to those found in the previous period.

Since there was no marked difference in the condition of the animal at the close of these respective lactation periods, it would seem reasonable to conclude that in this case the depression in apparent digestibility was not accompanied by a corresponding decrease in the productive efficiency of the ration.

## CONCLUSIONS

1. No marked difference in the digestibility of a very low protein ration was observed between cows long accustomed to the ration and others which had been recently transferred from a ration containing a liberal supply of protein.
2. The total consumption of water and digestibility of the ration were also unaffected by changing from well water to distilled water or vice versa.
3. The apparent digestibility of each ingredient of the ration, but more particularly of the ether extract, was much lower than the digestibility as computed by the use of average digestion coefficients.
4. In the one instance where a comparison is possible a considerable depression in the observed digestibility did not result in decreased efficiency of the ration.

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# THE BACTERIAL CONTENT OF ICE CREAM*

## A REPORT OF EXPERIMENTS IN BACTERIAL CONTROL IN SIX COMMERCIAL PLANTS

N. E. OLSON AND A. C. FAY

*Kansas Agricultural Experiment Station, Manhattan, Kansas*

## INTRODUCTION

The work herein reported is a continuation of the studies on bacterial control in ice cream. In a previous publication (1) it was shown that it was possible to produce consistently ice cream containing less than 100,000 bacteria per gram, and methods for producing ice cream of low bacterial count were prescribed. These methods have also been described in popular form in a special circular (2).

The work outlined in this paper was conducted with two purposes in mind, first, to learn what the average ice cream plant was doing in bacterial control; and second, to show the improvement which could be effected by following prescribed methods. It was also wished to strengthen further conclusions drawn in previous reports.

## PLAN OF EXPERIMENT

*Selection of plants.* Six ice cream plants were selected for the work. Selection of these plants was based on capacity, plant conditions, railway facilities, and type and quality of raw materials used.

*Methods.* The senior author supervised the work in the plants while the junior author handled the bacterial analysis at the station laboratory. Four days were spent in each plant. A mix was made each day, thus the experiments in each plant, with one exception, cover four mixes. No change was made during the first two days in the usual methods employed in the plant. The

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* Contribution No. 51 from Department of Dairy Husbandry and No. 73 from Department of Bacteriology. Received for publication April 16, 1925.

second two mixes in each plant were supervised, including the entire process from washing and sterilizing equipment to the freezing of the ice cream. Faulty methods noted during the first two days were corrected wherever possible.

*Sampling.* Milk thieves, glass stoppered sample bottles, spoons, and butter triers, were wrapped in paper, sterilized and shipped to the plants. The samples were packed in a regular ice cream packing can with ice and salt, and shipped to the laboratory by express. The samples consequently arrived at the laboratory in a frozen condition. Since all samples received the same treatment the results are comparative even though the freezing may have reduced the counts to some extent.

Samples were taken with sterile milk thieves except the samples "after homogenizing," "after cooling," and "after freezing" which were caught directly in sterile bottles. The "after hardening" sample was taken at the same time and in the same manner as the "after freezing" sample but was held in a frozen condition for an additional twenty-four hours. Samples of dry materials were taken with sterile spoons. Butter was sampled with a sterile trier and transferred to a bottle with a sterile spoon.

The ingredients were all sampled before adding them to the mixing vat. Owing to the presence of undissolved milk powder, gelatin, or butter it was not always possible to obtain a representative "before pasteurizing" sample before the mix reached a bacteria killing temperature. Therefore, to insure uniformity, all "before pasteurizing" counts are calculated from the counts on the ingredients used in preparing the mix (see tables 1 and 5). The "after pasteurizing" sample was obtained from the mixing vat at the time the homogenizer was started. Time and temperature records of pasteurization were taken from the moment the mix reached its maximum temperature until the homogenizer was started.

The "before homogenizing" sample was taken from the mixing vat after about three-quarters of the mix had run out. It required from thirty to seventy-five minutes for three-fourths of the mix to run through the homogenizer and since the temperature was maintained, that portion of the mix still in the vat when the "before homogenizing" sample was taken had in reality been subjected to pasteurizing temperature for an additional thirty to seventy-five minutes. This accounts for the frequent marked reduction in counts between the "after pasteurizing" and "before homogenizing" samples. This method was followed because it would give evidence of the minimum count which part of the mix reached and also made it possible to note the effect of homogenizing on the counts without the contamination which might take place from the homogenizer itself. The "after homogenizing" and "after cooler" samples were taken at approximately the same time as the "before homogenizing" sample, the former immediately after passing through the homogenizer and the latter immediately after passing over the cooler. The "after cooler" sample therefore does not indicate the true sanitary condition of the cooler, since considerable mix had washed over it and undoubtedly removed much of the contamination before the sample was taken. The "before aging" sample was taken from the aging vat after the entire mix had been cooled to aging temperature. It therefore included the contamination from the aging vat. The "after aging" sample was taken just before the first batch was run into the freezer.

The "after freezing" and "after hardening" samples were taken from the first batch of mix frozen in the cleaned freezer.

*Bacteriological methods.* The bacteriological analyses were made on a gravimetric basis. The purpose of using a gravimetric sample was to overcome the variation in volumetric samples of melted ice cream resulting from incomplete exhaustion of air, and the adherence of melted ice cream to the pipettes. In order to make the counts made at various stages comparable, ten grams were weighed under aseptic conditions for all of the various samples as well as the finished ice cream. The counts, therefore, represent the number of bacteria per gram. The determination of total numbers of bacteria was made according to the plate method recommended by the American Public Health Association. Liebig's beef extract and Difco peptone were used in the preparation of the agar.

## EXPERIMENTAL RESULTS

Since the conditions in the six plants were very different, no two employing identical methods, it seems more desirable to discuss each plant separately, later bringing all the results together in a summary. The plants are not listed in the order visited and, for obvious reasons, their identity is not disclosed.

### PLANT I

Plant I was a comparatively small plant manufacturing approximately 40,000 gallons of ice cream annually.

*Equipment.* A 200-gallon horizontal mixing vat,[1] a 100-gallon per hour homogenizer,[2] a tubular cooler, a 50-gallon starter can and a 40-quart horizontal brine freezer comprised the equipment. This apparatus was connected with sanitary pipe and fittings with the exception of the outlet from the mixing vat which was a galvanized iron pipe, globe valve combination. About 10 feet of galvanized iron pipe with a globe valve conveyed the mix from the starter can to the freezer. The mix was aged in 10-gallon milk cans.

*Raw materials.* The mix was compounded from the following materials: raw whole milk, received daily from the producers; unsalted butter; a fair grade of skimmilk powder; cane sugar; and a good grade of gelatin. Table 1 gives the bacterial counts on the ingredients used. The whole milk ranged in count from 1,100,000 to 5,200,000 bacteria per gram; the butter from 11,000 to 152,000 per gram; the skimmilk powder, 320,000; the gelatin, 13,000; and the sugar, 240 bacteria per gram.

*Cleaning and sterilizing methods.* The equipment was carelessly washed with hot water and washing powder and no effort was made to sterilize the vats and freezer. The homogenizer was not taken apart but was washed by pumping a part of the water from the vat through it and over the cooler. After the cooler had been left as clean, milky fluid was still visible in places on it.

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[1] This vat was also used for pasteurizing sour cream for buttermaking.

[2] The name "homogenizer" will be used to indicate all machines used for the purpose of homogenizing regardless of trade name.

TABLE 1

*The bacteria count per gram of the ingredients*

| PLANT NUMBER | MIX NUMBER | CREAM | WHOLE MILK | SKIM MILK | CONDENSED MILK | MILK POWDER | BUTTER | GELATIN | SUGAR | WATER |
|---|---|---|---|---|---|---|---|---|---|---|
| I | 1 | | 1,100,000 | | | 320,000 | 135,000 | 13,000 | 240 | |
| | 2 | | 1,730,000 | | | 320,000 | 152,000 | 13,000 | 240 | |
| | 3 | | 1,910,000 | | | 320,000 | 11,000 | 13,000 | 240 | |
| | 4 | | 5,200,000 | | | 320,000 | 20,200 | 13,000 | 240 | |
| II | 5 | 8,600,000 | | 6,650,000 | 272,000 | | | 7,000 | 180 | |
| | 6 | 2,400,635 | | 2,880,000 | 91,000 | | | 7,000 | 180 | |
| | 7 | 4,230,000 | | 1,740,000 | 91,000 | | | 7,000 | 180 | |
| | 8 | 3,230,000 | | 314,000 | 1,160,000 | | | 7,000 | 180 | |
| III | 9 | 580,000 | 60,000,000 | | | 418,000 | | 550 | 20 | |
| | 10 | 4,170,000 | 22,000,000 | | | 418,000 | | 550 | 20 | |
| | 11 | 555,000 | 11,800,000 | | | 418,000 | | 550 | 20 | |
| | 12 | 1,860,000 | 22,900,000 | | | 418,000 | | 550 | 20 | |
| IV | 13 | 975,000 | 23,314,000 | | 11,800,000 | | | 13,400 | 120 | |
| | 14 | 1,110,000 | 56,800,000 | | 15,400,000 | | | 91,000 | 120 | |
| | 15 | 168,000 | 18,500,000 | | 60,000 | | | 91,000 | 120 | |
| | 16 | | 3,490,000 | | 7,600 | | 15,500 | 91,000 | 90 | |
| V | 17 | 374,000 | 750,000 | | 166,000 | | | 800 | 70 | 16 |
| | 18 | 5,590,000 | 6,100 | | 143,000 | | | 800 | 70 | 16 |
| | 19 | 1,240,000 | 15,800 | | 2,390,000 | | | 800 | 70 | |
| VI | 20 | 9,930,000 | 1,280,000 | | | 160,000 | 280,000 | 11,300 | 180 | 245 |
| | 21 | 30,400,000 | 5,960,000 | | | 160,000 | 1,810,000 | 11,300 | 180 | 245 |
| | 22 | 6,900,000 | 2,750,000 | | | 20,000 | 290,000 | 11,300 | 20 | 245 |
| | 23 | 32,000,000 | 3,660,000 | | 92,300 | 20,000 | 211,000 | 11,300 | 20 | 245 |
| Average......... | | 6,350,702 | 12,798,205 | 2,896,000 | 2,639,408 | 276,000 | 324,966 | 18,095 | 122 | 168 |

TABLE 2

*The number of bacteria per gram of ice cream mix at various stages in the manufacturing process*

| PLANT | MIX | PASTEURIZATION |  |  |  |  | HOMOGENIZING |  | AFTER COOLING | AGING |  | AFTER FREEZING | AFTER HARDENING |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  | Time | Temperature | Before (calculated) | After | Per cent efficiency | Before | After—before cooling |  | Before | After |  |  |
| I | 1 | 30 | 147 | 797,615 | 4,300 | 99.461 | 580 | 1,100 | 12,600 | 89,000 | 81,000 | 320,000 | 145,000 |
|  | 2 | 30 | 145 | 1,237,143 | 4,600 | 99.629 | 2,000 | 750 | 2,700 | 30,000 | 290,000 | 332,000 | 265,000 |
|  | 3 | 30 | 147 | 1,372,810 | 2,250 | 99.837 | 1,900 | 3,000 | 1,700 | 8,200 | 16,500 | 118,000 | 91,000 |
|  | 4 | 30 | 150 | 3,701,609 | 900 | 99.976 | 460 | 830 | 900 | 4,000 | 4,300 | 98,000 | 32,500 |
| II | 5 | 0 | 145 | 5,411,512 | 546,000 | 89.911 | 231,000 | 875,000 | 834,000 | 671,000 | 700,000 | 688,000 | 769,000 |
|  | 6 | 20 | 142 | 4,615,657 | 123,000 | 97.357 | 208,000 | 398,000 | 441,000 | 385,000 | 692,000 | 660,000 | 546,000 |
|  | 7 | 30 | 145 | 3,206,057 | 66,900 | 96.969 | 19,500 | 30,300 | 62,000 | 72,200 | 74,800 | 70,000 | 39,000 |
|  | 8 | 30 | 150 | 1,553,257 | 28,000 | 98.198 | 22,700 | 16,300 | 20,000 | 43,500 | 28,500 | 23,700 | 6,200 |
| III | 9 | 16 | 145 | 34,657,705 | 10,700 | 99.970 | 14,300 | 17,000 | 16,000 | 31,000 | 39,000 | 84,000 | 100,000 |
|  | 10 | 15 | 145 | 13,713,505 | 3,000 | 99.980 | 3,100 | 3,000 | 4,300 | 9,700 | 12,400 | 56,000 | 48,000 |
|  | 11 | 30 | 147 | 6,939,288 | 2,200 | 99.968 | 1,600 |  |  | 2,300 | 2,800 | 4,400 | 3,200 |
|  | 12 | 30 | 150 | 13,277,805 | 57,000 | 99.570 | 10,000 | 21,500 |  | 21,100 | 21,900 | 22,000 | 17,000 |
| IV | 13 | 5 | 145 | 15,520,085 | 868,000 | 94.400 | 322,000 | 227,000 | 443,000 | 244,000 | 528,000 | 1,507,000 | 1,253,000 |
|  | 14 | 40 | 145 | 35,273,679 | 850,000 | 97.591 | 325,000 | 401,500 | 474,000 | 89,000 | 98,000 | 275,000 | 238,000 |
|  | 15 | 30 | 150 | 10,768,303 | 125,000 | 99.583 | 45,000 | 54,000 | 18,500 | 80,000 | 88,000 | 97,000 | 57,000 |
|  | 16 | 30 | 145 | 2,164,758 | 29,200 | 98.652 | 3,900 | 5,300 | 4,900 | 13,400 | 20,000 | 64,000 | 73,000 |

TABLE 2—*Continued*

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V | 17 | 20 | 165 | 430,900 | 2,000 | 99.536 | 2,100 | 8,200 | | 29,700 | 13,000 | 17,400 | 47,600 |
| | 18 | 20 | 165 | 1,755,011 | 2,100 | 99.880 | 2,600 | 2,400 | | 3,500 | 8,200 | 10,500 | 11,100 |
| | 19 | 20 | 165 | 804,868 | 4,500 | 99.441 | 19,400 | 39,500 | | 50,800 | 70,000 | 102,000 | 79,000 |
| VI | 20 | 0 | 144 | 2,916,086 | 500,000 | 82.854 | 30,300 | 80,000 | 70,300 | 940,000 | 910,000 | 950,000 | 680,000 |
| | 21 | 0 | 145 | 12,773,900 | 2,730,000 | 78.629 | 128,000 | 395,000 | 368,000 | 950,000 | 880,000 | 600,000 | 580,000 |
| | 22 | 30 | 149 | 2,760,647 | 21,500 | 99.221 | 2,800 | 7,600 | | 30,800 | 27,000 | 26,400 | 17,500 |
| | 23 | 30 | 148 | 4,243,488 | 58,800 | 98.704 | 3,100 | 8,100 | | 18,900 | 11,700 | 14,000 | 15,000 |
| Average | | | | 7,821,551 | 262,606 | 96.643 | 60,840 | 117,971 | 173,368 | 165,970 | 200,743 | 266,947 | 222,308 |

The sanitary piping was slightly steamed. The cans in which the mix was collected from the cooler were washed in a vat of water which was not changed often enough and were then set over a steam jet for a few seconds, after which they were inverted on a rack. A stale odor was noticeable in most of the cans when taken down for use.

*Processing the mix.* The skimmilk powder, gelatin and sugar were added to the cold milk in the vat and heat applied at once. The butter was then added in about one pound pieces. When the mix reached pasteurizing temperature, which for this plant was 145°F., the ingredients were in solution or suspension. The mix was held at 145° for thirty minutes, after which it was homogenized without cooling, so that the last part of the mix was at bacteria killing temperatures for approximately one hour longer. After homogenizing, the mix was cooled to 66°F. by passing over the tubular cooler, and was collected in 10-gallon cans. The mix was spread on the cooler with a bottle brush which had been used in washing cans. The first can of mix was allowed to remain in the warm room until the last can was ready, after which they were placed, without further cooling or agitation, in a cold room at 38°F. Here they remained until the following morning when four or five cans were removed at a time, dumped into an elevated starter can, and cooled with brine to 40°F. preparatory to freezing. The average temperature of the mix when taken from the cold rooms was 50°F. From the starter can the mix flowed by gravity through a globe valve and galvanized iron pipe to the freezer.

## *Results*

*Unsupervised mixes.* An examination of the data in table 2, mixes 1 and 2, will show a moderately high original count; over 99 per cent efficiency of pasteurization; and a normal increase due to homogenization. The counts from mix 1 indicate contamination from an improperly cleansed tubular cooler. It is also worthy of note that about 130 gallons of mix had washed over the cooler when the sample was taken.

No aging vat was available and since it was impractical to follow 15 individual cans of mix through the entire process, the "before aging" sample was obtained from a composite taken from four cans by means of a sterile glass milk thief. The first, fifth, tenth, and fifteenth cans were selected as representative. Individual samples were also taken from each can and the counts are shown in table 3.

The figures in table 3 indicate that the cooler must have been responsible for much of the increase in numbers noted in the "before aging" sample, since the count of the first can increased from 4300 to 325,000. By the time the fifth, tenth, and fifteenth cans of mix had passed over the cooler the greater part of the contamination presumably had been washed off. It is probable that part of the increase noted above is due to the improperly sterilized cans.

TABLE 3

*Contamination due to cooler*

| AFTER PASTEURIZING | FIRST CAN | FIFTH CAN | TENTH CAN | FIFTEENTH CAN | BEFORE HOMOGENIZING |
|---|---|---|---|---|---|
| 4,300 | 325,000 | 24,000 | 7,800 | 4,600 | 580 |
| | SAME CANS AFTER AGING | | | | |
| | 185,000 | 31,200 | 71,000 | 34,300 | |

Referring to table 3 it is noted that the counts on cans 5, 10 and 15 show a considerable increase after aging. Because of the manner in which the cans of mix were handled this increase was not unexpected. Can 1, however, shows a marked decrease probably due to experimental error, and as a result the averages recorded in table 2 show a slight decrease. That one of these counts is in error is further strengthened by the fact that the second day's mix (no. 2), produced under identical conditions, actually shows an increase from 30,000 to 290,000 instead of a decrease.

A poorly washed freezer further increased the counts while hardening resulted in a decrease.

*Supervized mixes; methods and results.* The second two mixes studied at plant I (table 2, mixes 3 and 4) show the results of improved methods of cleaning and proper cooling. In table 4 are recorded the averages for each two day period.

TABLE 4

*The average counts for the first two days without supervision and for the second two days under supervision*

| PLANT NUMBER | DAYS | PASTEURIZATION | | | HOMOGENIZATION | | AFTER COOLING | AGING | | AFTER FREEZING | AFTER HARDENING |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Before | After | Per cent efficiency | Before | After | | Before | After | | |
| I | First two days | 1,016,879 | 4,450 | 99,563 | 1,290 | 925 | 7,650 | 59,500 | 185,500 | 326,000 | 205,000 |
| | Second two days | 2,537,210 | 1,575 | 99.938 | 1,180 | 1,915 | 1,300 | 6,100 | 10,400 | 108,000 | 61,750 |
| II | First two days | 5,013,584 | 332,500 | 93.369 | 219,500 | 636,500 | 637,500 | 528,000 | 696,000 | 674,000 | 657,500 |
| | Second two days | 2,379,657 | 47,450 | 98.007 | 21,100 | 23,300 | 41,000 | 57,850 | 51,650 | 46,850 | 22,600 |
| III | First two days | 24,185,605 | 6,850 | 99.972 | 8,700 | 10,000 | 10,150 | 20,450 | 25,700 | 70,000 | 74,000 |
| | Second two days | 10,108,546 | 29,200 | 99.712 | 5,800 | | | 11,700 | 12,350 | 13,200 | 10,100 |
| IV | First two days | 25,396,882 | 859,000 | 96.618 | 323,500 | 314,250 | 458,500 | 166,500 | 313,000 | 891,000 | 745,500 |
| | Second two days | 6,466,530 | 77,100 | 98.808 | 24,450 | 29,650 | 11,700 | 46,700 | 54,000 | 80,500 | 65,000 |
| V | First two days | 1,092,955 | 2,050 | 99.813 | 2,350 | 5,300 | | 16,600 | 10,600 | 13,950 | 29,350 |
| | Third day | 804,868 | 4,500 | 99.441 | 19,400 | 39,500 | | 50,800 | 70,000 | 102,000 | 79,000 |
| VI | First two days | 7,844,993 | 1,615,000 | 79.414 | 79,150 | 237,500 | 219,150 | 945,000 | 895,000 | 775,000 | 630,000 |
| | Second two days | 3,502,067 | 40,150 | 98.854 | 2,950 | 7,850 | | 24,850 | 19,350 | 20,200 | 16,250 |
| Average... | First two days | 10,758,566 | 470,308 | 95.629 | 105,748 | 200,745 | 266,590 | 289,341 | 354,300 | 458,325 | 390,225 |
| | Second two days | 4,617,535 | 36,022 | 99.220 | 11,850 | 18,643 | 18,000 | 31,381 | 33,227 | 58,136 | 39,127 |

Since steam was not available for sterilizing purposes hot water was used. In the morning before making the mix the vat was filled one-third full of water and with the covers tightly closed was heated by means of the revolving coil to 180°F. This hot water was then pumped by means of the homogenizer through the piping and over the cooler, using a clean brush to spread the water over the cooler. More care was also observed in steaming the cans. The ice cream maker was instructed to wash the starter can and freezer by a similar method, since the supervisor could not be present.

No changes were recommended in the handling of the mix with the exception of cooling. After all the mix had passed over the cooler it was dumped back into the mixing vat and cooled to 40°F. with circulating ice water. The mix was then drawn off into the original cans, which were placed in the cold room (38°F.) for twenty-four hours. While this was a laborious method of handling the mix it demonstrated the importance of proper cooling as a factor in bacterial control.

Again referring to the data in tables 2 and 4 it is evident that exceedingly efficient pasteurization and comparative freedom from equipment contamination were attained, the average count "before aging" being only 6100 bacteria per gram for the second two days as compared to 59,500 bacteria per gram the first two days. The results from mix 3 show an increase from 8200 to 16,500 during aging while in mix 4 the increase is only from 4000 to 4300. Probably the increase in no. 3 during aging should be checked to experimental error since the aging temperature was as low as in mix 4.

An abnormal increase is noted in both mixes 3 and 4 "after freezing" probably due to failure on the part of the ice cream maker to wash the freezer as instructed.

The average final counts for the two days with supervision was 61,750 bacteria per gram compared to 205,000 bacteria per gram without supervision.

## PLANT II

Plant II manufactured about 10,000 gallons of ice cream annually. The mixes averaged about 2000 pounds.

*Equipment.* A rather unusual and therefore very interesting condition was found in plant II, it being the only plant studied in which galvanized iron pipe and fittings and globe valves were used exclusively for conveying the mix. The equipment included a 300-gallon horizontal mixing vat, two 300-gallon aging vats, a 200-gallon per hour homogenizer, a tubular cooler, two small pumps, and two 40-quart horizontal brine freezers. A 200-gallon vat and a small tubular cooler were used for pasteurizing and cooling sweet cream.

*Raw materials.* The sweet cream used was received from the producers in small amounts and was of uncertain quality. The cream was heated to about 125° to 130°F. for a few minutes and then cooled in order to keep the cream sweet until ready to make the mix. The raw cream ranged from 31 million to 50 million bacteria per gram before heating and from 2,400,000 to 8,600,000 bacteria per gram after heating. The skimmed milk used was purchased from a local milk pasteurizing plant and was supposed to have been pasteurized. The counts ranged from 214,000 to 6,650,000 bacteria per gram. The counts on the plain condensed skimmed milk used varied between 91,000 and 1,160,000 bacteria per gram. The gelatin contained 7000 bacteria per gram and the cane sugar 180 bacteria per gram.

*Cleaning and sterilizing methods.* Since the piping was all of galvanized iron with globe valves, it was not practical to disconnect it for cleaning. The mix conveying pipes were fitted with water and steam connections. After use, cold water was run through the pipes lines followed by hot water and finally by steam under slight pressure. The homogenizer and pumps were not taken apart but, while still running, were washed and steamed in the same manner as the piping. After the vats were washed with warm water, but without washing powder, they were steamed until the temperature exceeded 200°F. The entire system was again steamed before starting the mix the next morning. The tubular cooler was rather carelessly washed, the use of insufficient water and brushing leaving a milky fluid to dry between the tubes. The mix was pumped from the aging vat to the freezers. As previously mentioned, the pump was not taken apart for cleaning.

The freezers were well washed with an abundance of hot water and steamed for five minutes.

*Processing the mix.* The entire mix was pasteurized. It was evident that the workman had the wrong conception of what was meant by pasteurization at 145°F. for thirty minutes, since the homogenizer was started as soon as the mix reached 145°F. After the mix had been running through the homogenizer for thirty minutes, that remaining in the vat was cooled to 120°F. In mix 6 the aging vat was not ready to receive the mix, consequently it was held at an average of 142°F. for twenty minutes and cooled to 110°F. after the homogenizer had been running ten minutes.

From the homogenizer the mix was conveyed through about 30 feet of piping to the tubular cooler where it was cooled to 62°F. Cooling to 35°F. was completed in the vat. The temperature did not exceed 45°F. during aging. The aging period was eighteen to twenty hours.

### *Results*

*Unsupervised mixes.* Since pasteurizing was carelessly handled the efficiency was low; namely, 89.91 and 97.35 per cent respectively in the two mixes studied. The increases in bacterial count recorded in table 2 at certain stages in the processing indicate considerable contamination from equipment. But for the nature of the piping and the fact that there were traps in the lines to which the water and steam could not gain access, one would expect, considering the methods employed in steaming, freedom from contamination. Since the freezers were properly washed the average counts "after aging," 696,000, and "after freezing," 674,000 show no increase at this point.

It sometimes happened that one aging vat was full and the other only partly emptied when quitting time came. As a result it was impractical to wash the conveying pipe leading from the vat. Such a condition occurred while the supervisor was present. The mix in vat 2 was not all frozen when it came time to quit. The vat valve was closed. Cold water was then admitted to the line and pumped through the freezers. This was followed with

TABLE 5

*Showing the per cent of the total count due to each ingredient and the per cent composition of the mix*

| PLANT NUMBER | MIX NUMBER | CREAM | | WHOLE MILK | | SKIM MILK | | CONDENSED MILK | | MILK POWDER | | BUTTER | | GELATIN | | SUGAR | | WATER | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Per cent composition | Per cent bacteria count | Per cent composition | Per cent bacteria count | Per cent composition | Per cent bacteria count | Per cent composition | Per cent bacteria count | Per cent composition | Per cent bacteria count | Per cent composition | Per cent bacteria count | Per cent composition | Per cent bacteria count | Per cent composition | Per cent bacteria count | Per cent composition | Per cent bacteria count |
| I | 1 | | | 69.69 | 96.11 | | | | | 4.90 | 1.96 | 11.28 | 1.90 | 0.40 | 0.006 | 13.73 | 0.003 | | |
| | 2 | | | 69.60 | 97.33 | | | | | 4.90 | 1.26 | 11.27 | 1.38 | 0.49 | 0.005 | 12.72 | 0.002 | | |
| | 3 | | | 71.00 | 98.77 | | | | | 4.84 | 1.12 | 11.15 | 0.09 | 0.40 | 0.003 | 12.61 | 0.002 | | |
| | 4 | | | 70.84 | 99.52 | | | | | 4.71 | 0.40 | 11.32 | 0.06 | 0.37 | 0.001 | 12.73 | 0.0008 | | |
| II | 5 | 33.36 | 53.02 | | | 37.60 | 46.20 | 15.16 | 0.76 | | | | | 0.40 | 0.0006 | 13.43 | 0.0004 | | |
| | 6 | 33.05 | 76.66 | | | 36.88 | 23.01 | 16.26 | 0.32 | | | | | 0.48 | 0.0007 | 13.32 | 0.0005 | | |
| | 7 | 38.24 | 73.31 | | | 33.01 | 26.00 | 14.94 | 0.67 | | | | | 0.48 | 0.001 | 13.34 | 0.001 | | |
| | 8 | 39.40 | 81.95 | | | 30.96 | 6.25 | 15.76 | 11.78 | | | | | 0.47 | 0.002 | 13.39 | 0.001 | | |
| III | 9 | 25.00 | 0.41 | 57.50 | 99.54 | | | | | 3.04 | 0.03 | | | 0.37 | 0.000005 | 14.06 | 0.000008 | | |
| | 10 | 25.02 | 7.61 | 57.53 | 92.30 | | | | | 2.99 | 0.09 | | | 0.37 | 0.00001 | 14.09 | 0.00002 | | |
| | 11 | 25.03 | 2.00 | 57.58 | 97.82 | | | | | 3.00 | 0.18 | | | 0.30 | 0.00003 | 14.08 | 0.00003 | | |
| | 12 | 26.56 | 3.72 | 55.77 | 96.19 | | | | | 3.07 | 0.09 | | | 0.37 | 0.00001 | 14.19 | 0.00002 | | |
| IV | 13 | 18.48 | 1.17 | 58.91 | 88.34 | | | 13.77 | 10.47 | | | | | 0.55 | 0.0005 | 8.26 | 0.00006 | | |
| | 14 | 19.57 | 0.61 | 57.98 | 93.36 | | | 13.78 | 6.01 | | | | | 0.52 | 0.001 | 8.13 | 0.00002 | | |
| | 15 | 19.57 | 0.30 | 57.98 | 99.61 | | | 13.78 | 0.07 | | | | | 0.52 | 0.003 | 8.13 | 0.00008 | | |
| | 16 | | | 61.93 | 99.92 | | | 12.45 | 0.04 | | | 10.08 | 0.07 | 0.55 | 0.02 | 14.23 | 0.0005 | | |

TABLE 5—*Continued*

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V | 17 | 32.15 | 27.91 | 37.50 | 65.28 | | | 17.64 | 6.77 | | | | | 0.45 | 0.0006 | 10.29 | 0.001 | 1.96 | 0.00006 |
| | 18 | 30.87 | 98.34 | 38.43 | 0.13 | | | 18.63 | 1.52 | | | | | 0.44 | 0.0002 | 10.35 | 0.0004 | 1.25 | 0.00001 |
| | 19 | 27.97 | 43.09 | 42.20 | 0.81 | | | 18.88 | 56.07 | | | | | 0.40 | 0.0004 | 10.47 | 0.0008 | | |
| VI | 20 | 26.68 | 90.87 | 19.62 | 8.61 | | | | | 5.73 | 0.31 | 1.96 | 0.18 | 0.62 | 0.002 | 14.28 | 0.0008 | 31.08 | 0.002 |
| | 21 | 39.35 | 93.66 | 12.38 | 5.76 | | | | | 6.25 | 0.07 | 3.47 | 0.49 | 0.61 | 0.0005 | 14.05 | 0.0002 | 23.86 | 0.0004 |
| | 22 | 22.65 | 65.62 | 34.36 | 34.23 | | | | | 5.64 | 0.04 | 0.96 | 0.10 | 0.62 | 0.002 | 14.05 | 0.0001 | 18.11 | 0.001 |
| | 23 | 11.03 | 83.20 | 18.51 | 15.95 | | | 16.97 | 0.37 | 2.62 | 0.01 | 9.14 | 0.45 | 0.50 | 0.001 | 7.33 | 0.00004 | 33.87 | 0.0002 |

TABLE 6

*Bacteria added to each gram of mix by various ingredients*

| PLANT NUMBER | MIX NUMBER | CREAM | WHOLE MILK | SKIM MILK | CONDENSED MILK | MILK POWDER | BUTTER | GELATIN | SUGAR | WATER | TOTAL COUNT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 1 | | 766,640 | | | 15,700 | 15,200 | 50 | 25 | | 797,615 |
| | 2 | | 1,204,225 | | | 15,686 | 17,137 | 63 | 32 | | 1,237,143 |
| | 3 | | 1,356,000 | | | 15,500 | 1,230 | 50 | 30 | | 1,372,810 |
| | 4 | | 3,684,150 | | | 15,094 | 2,286 | 49 | 30 | | 3,701,609 |
| II | 5 | 2,869,700 | | 2,500,500 | 41,255 | | | 33 | 24 | | 5,411,512 |
| | 6 | 3,538,800 | | 1,062,000 | 14,800 | | | 33 | 24 | | 4,615,657 |
| | 7 | 1,618,000 | | 574,000 | 14,000 | | | 33 | 24 | | 3,206,057 |
| | 8 | 1,273,000 | | 97,200 | 183,000 | | | 33 | 24 | | 1,553,257 |
| III | 9 | 145,000 | 34,500,000 | | | 12,700 | | 2 | 3 | | 34,657,705 |
| | 10 | 1,044,000 | 12,657,000 | | | 12,500 | | 2 | 3 | | 13,713,505 |
| | 11 | 138,750 | 6,788,125 | | | 12,409 | | 2 | 2 | | 6,939,288 |
| | 12 | 494,000 | 12,771,000 | | | 12,800 | | 2 | 3 | | 13,277,805 |
| IV | 13 | 183,000 | 13,711,000 | | 1,626,000 | | | 75 | 10 | | 15,520,085 |
| | 14 | 217,319 | 32,933,734 | | 2,122,138 | | | 479 | 9 | | 35,273,679 |
| | 15 | 32,891 | 10,726,656 | | 8,268 | | | 479 | 9 | | 10,768,303 |
| | 16 | | 2,161,617 | | 946 | | 1,674 | 509 | 12 | | 2,164,758 |
| V | 17 | 120,300 | 281,300 | | 29,290 | | | 3 | 7 | 0.3 | 430,900 |
| | 18 | 1,726,000 | 2,300 | | 26,700 | | | 4 | 7 | 0.2 | 1,755,011 |
| | 19 | 346,861 | 6,668 | | 451,328 | | | 4 | 7 | | 804,868 |
| VI | 20 | 2,650,077 | 251,177 | | | 9,167 | 5,494 | 70 | 25 | 76 | 2,916,086 |
| | 21 | 11,964,478 | 736,370 | | | 10,000 | 62,900 | 69 | 25 | 58 | 12,773,900 |
| | 22 | 1,811,600 | 945,000 | | | 1,130 | 2,800 | 70 | 3 | 44 | 2,760,647 |
| | 23 | 3,530,864 | 677,000 | | 15,668 | 524 | 19,292 | 56 | 1 | 83 | 4,243,488 |
| Average........ | | 1,872,480 | 7,166,313 | 1,058,425 | 377,782 | 11,100 | 14,223 | 94 | 14 | 43 | 7,821,551 |

warm water and then given a thorough steaming. It is at once evident that part of the line could not be cleaned. Also the steam heated the mix in the vat behind the valve. The following morning a sample was taken from vat two after agitating and then a five gallon batch was pumped into the receiving tank on the freezer where another sample was taken after stirring with a sterile milk thief. These counts were respectively 360,000 and 506,000 bacteria per gram, which means that the first quart of mix pumped through the line carried contamination to the extent of approximately 3 million bacteria per gram.

*Supervised mixes: methods and results.* Extraordinary efforts were made on the supervised mixes (table 2, mixes 7 and 8) to reduce the counts obtained in the unsupervised work. A careful check of contaminating traps in the pipe lines was made. The pipe lines and cooler were flushed with water containing washing powder and this was followed with a considerable volume of hot water. The usual methods of steaming were used. The homogenizer was taken apart, cleaned and re-assembled when wanted for use.

Mix 7 was pasteurized at 145°F. for thirty minutes and mix 8 at 150°F. for thirty minutes before the homogenizer was started. Homogenization was performed at the pasteurizing temperature.

The average pasteurizing efficiency on the supervised mixes was 98 per cent. The reason for this efficiency not exceeding 99 per cent is probably due to the previous, though inefficient, pasteurization of the cream and skimmed milk. Contamination from equipment was slight and the final average count of the hardened ice cream was 22,600 bacteria per gram as compared to 657,500 bacteria per gram on the previous two day test.

The results in plant II demonstrate that it is possible, though difficult, to produce ice cream with a low bacterial content even where galvanized iron piping and globe valves are used. However, considering the danger of high counts inherent in such piping it cannot be recommended. There is also a possibility that under some conditions galvanized iron piping might be a contributing factor in the production of metallic flavors.

### PLANT III

Plant III was a comparatively small plant in rather poor condition.

*Equipment.* A 200-gallon horizontal mixing vat, an 80-gallon homogenizer, two 200-gallon aging vats, a champion cooler, and a horizontal brine freezer comprised the equipment. The mix was conveyed through sanitary piping. The lining of the mixing vat was in poor condition and the coil was badly warped due to freezing. There was a broken gauge on the homogenizer.

*Raw materials.* The mix in this plant was made from fresh milk and sweet cream with a small amount of skimmilk powder to balance the solids. The whole milk was of questionable quality averaging 30,675,000 bacteria per gram. Most of the milk was warm when received. The sweet cream was skimmed from a part of the whole milk, pasteurized, and used the following day, the counts then ranging from 555,000 to 4,170,000 bacteria per gram. The skimmilk powder contained 418,000; the gelatin 550, and the sugar 20 bacteria per gram.

*Methods of cleaning and sterilizing.* The method of washing at the end of the day was as follows: The vat was washed with warm water but no washing powder. This water was pumped through the homogenizer and sanitary piping. The piping was not brushed or taken down. The freezer was washed in a similar manner. Nothing except the sanitary piping was steamed and it only slightly. An examination of the equipment after it was supposedly clean and ready for use revealed the presence of a green scum on sanitary fittings and valves, and the walls and coil in the mixing vat were found to be greasy.

In the morning, before using, the homogenizer was taken apart and flushed out with water. After re-assembling, about 2 quarts of a hypochlorite solution was pumped through the homogenizer and piping, over the cooler, and into the aging vat. The vat gate was left open so the hypochlorite solution drained out immediately. Hypochlorite solution was also splashed onto the sides of the vat with a bottle brush.

*Processing.* The mix, with the exception of the sugar, was pasteurized at 145°F. for fifteen minutes, cooled to 120°F. and homogenized. There was no water in the champion cooler, it being used simply as a part of the conveying system. From this cooler the mix passed into the aging vat through a very rusty funnel, containing a piece of greasy copper screen. The sugar was added in the aging vat and the mix cooled to 35°F. The mix was usually aged about twenty to twenty-four hours.

### *Results*

*Unsupervised mixes.* The results secured in this plant were rather a surprise. They show that insanitary conditions and careless pasteurizing do not always mean high counts, although they usually do.

The average count of the mix before pasteurization (table 4) was 24,185,605 bacteria per gram. After pasteurization, the count was 6850; 99.972 per cent efficiency being obtained with a temperature of 145°F. for only fifteen minutes. As previously noted, the cream and milk were of very poor quality and were handled under poor conditions. The failure to keep the cream and milk cool, together with the warm weather which prevailed were conducive to a bacterial flora dominated by organisms of the colon group. Although no determination of the number of organisms of the colon type was made, it was noted that the predominating colonies on the plates were characteristic of colon colonies. Since the organisms of this group are very easily killed by heat it may account, in part at least, for the unexpected reduction in bacteria as a result of the relatively inefficient method of pasteurization. It has been a common observation in this and in previous work that exceptionally high counts in the raw mix are frequently accompanied by nearly 100 per cent efficiency of pasteurization, and in nearly all instances the conditions are indicative of a high colon content.

*Supervised mixes.* Before the supervised mixes were made the cooler, funnel, and copper screen were discarded and the piping was rearranged to facilitate cleaning. After washing, all equipment, including the freezer, was thoroughly steamed. The homogenizer was taken apart and left apart until ready to use the next day. After re-assembling, the hypochlorite solution was run through as before.

Pasteurization was changed from 145°F. for fifteen minutes to 150°F. for thirty minutes, and the homogenization was carried out at the pasteurizing temperature. The entire mix including the sugar was pasteurized.

The results (table 4, plant III) show a slightly lower pasteurizing efficiency than for the unsupervised mixes. The samples taken at the various stages show very little increase and the final count on the finished product was 10,100 bacteria per gram as compared to 74,000 in the unsupervised mixes.

### PLANT IV

This plant was the largest one included in the experiments, the usual size mix being 4300 pounds.

*Equipment.* The equipment, consisting of a 400-gallon mixing vat, a 300-gallon per hour homogenizer, two 400-gallon aging vats, and a tubular cooler, was in excellent condition. There were about 30 feet of sanitary pipe and fittings between the mixing and aging vat and about 20 feet more from the aging vats to two horizontal brine freezers located on a lower floor.

*Raw material.* The whole milk used was fresh and slightly better in quality than that used in plant III. The cream was pasteurized cream which had been held at 34°F. for twenty-three to twenty-five days. The cream had an old, slightly bitter flavor and a bacterial content of 168,000 to 1,110,000 bacteria per gram. Mixes 13 and 14 were made from the same batch of cream. Mix 15 was made from cream several days older than above but lower in count, while in mix 16 no sweet cream was used. The unsalted butter used in mix 16 was freshly churned and of good quality. The sweetened condensed milk used in mixes 13 and 14 gave a very high count; viz., 11,800,000 and 15,400,000 bacteria per gram respectively. The sweetened condensed milk used in mix 15 contained 60,000, while the plain condensed skimmilk (a very sandy 40 per cent solids product) contained only 7600 bacteria per gram. The gelatin used the first day contained 13,400 bacteria per gram, while that used the last three days contained 91,000.

*Methods of cleaning and sterilizing.* The equipment was first rinsed with luke warm water and then washed with hot water (170° to 180°F.), washing powder and brushes. No steam was used for sterilizing, but instead the entire system was given a final rinse with about 50 gallons of hot water (170° to 180°F.). This rinse was repeated in the morning before making the mix. No attempt was made to sterilize the freezer. Water was allowed to stand in the lower trough of the cooler. The homogenizer was taken apart for washing.

*Processing the mix.* The entire mix was pasteurized. The pasteurizing in this plant was also rather uncertain, being 145°F. for five minutes on the first day and 145°F. for forty minutes on the second day. The longer time on the second day was not intentional but was due to the fact that the aging vat was not ready to receive the mix. The mix was homogenized at the pasteurizing temperature, cooled over the tubular cooler to 80°F., and further cooled with brine to 35°F. in the aging vat. During aging the temperature of the mix did not exceed 45°F. The mix was usually aged about twenty-four hours.

## *Results*

*Unsupervised mixes.* An examination of the data secured from mixes 13 and 14 (table 2) reveals very high initial counts, 15,520,085 and 35,270,679 respectively. The efficiency of pasteurizing was 94.4 and 97.59 per cent, leaving an after pasteurizing count of approximately 850,000 bacteria per gram in each case. From this point on the counts were erratic but gave constant increases, especially between the "after aging" and "after freezing" samples, which increased from 528,000 to 1,507,000 in mix 13, and from 98,000 to 257,000 in mix 14, indicating considerable contamination from the freezer.

In connection with mixes 13 and 14 the following explanation is of interest. On the day the first experiment was started it was found that a mix was already in the process of preparation. Since another mix was to be made as soon as the first was finished, the second mix was chosen for the experiment. However, one sample was taken from the aging vat of the first mix. The count on this mix proved to be very low; viz., 2400 bacteria per gram.

The second mix was prepared without washing the equipment, the maker reasoning that since all the material which had just gone through the system was of a good quality there was no need for washing. The bacterial counts on the second mix were very high due to "pin point" colonies, presumably thermophilic bacteria (3). Similar results have been noted in the college ice cream laboratory. While the evidence is not conclusive it seems logical to believe that thermophilic bacteria are frequently present, though in such small numbers as to escape notice. Ordinarily thirty minutes pasteurization is insufficient time for noticeable growth. However, in this instance it is probable that growth began in the first mix and, since the equipment was not washed, resulted in heavy contamination of the second mix. With this heavy contamination noticeable growth was possible during the pasteurization of the succeeding mix. Since the same type of colony was also present in the mix made on the following day (no. 14) the wash water apparently was not hot enough to destroy the contamination. The inconsistency in the counts on these two mixes was due to the fact that the plates were crowded with these small "pin point" colonies, which rendered accurate determinations difficult to obtain.

*Supervised mixes.* All the equipment was thoroughly steamed in addition to the usual method of washing. As a result mix 15 contained considerably fewer "pin point" colonies. The equipment was again thoroughly steamed before mix 16 was prepared. The culture plates made from mix 16 were entirely free from "pin point" colonies.

Mixes 15 and 16 were pasteurized at 150° and 145°F. for thirty minutes, respectively. The initial counts in these mixes were lower than in nos. 13 and 14. The pasteurizing efficiency was high and contamination from equipment fairly low. The average final count on the second two days was 65,000 bacteria per gram as compared to an average of 745,500 bacteria per gram for the first two days.

This experience furnishes further proof that even under extremely difficult conditions it is possible to produce ice cream of low bacterial count if thorough cleaning and sterilizing methods are used, and where pasteurization is carried on in accordance with the Kansas law. The statutes of Kansas stipulate that all dairy products used in the manufacture of ice cream be pasteurized at not less than 145°F. for not less than thirty minutes.

### PLANT V

This was one of the larger plants visited. The mixes averaged about 3000 pounds.

*Equipment.* The equipment consisted of a 400-gallon horizontal pasteurizing vat, a 300-gallon per hour homogenizer, a 400-gallon vertical glass enameled aging tank, and two 40-quart horizontal brine freezers. There were about 100 feet of sanitary piping between the mixer and the aging tank and about fifteen feet between the aging tank and the freezers.

*Raw materials.* The fresh milk and cream used at this plant were much lower in bacterial counts than that received at the other plants visited. The cream was pasteurized at an outlying station and shipped to the ice cream plant. The cream averaged 2,401,000 bacteria per gram. The milk, surplus from a bottling plant, averaged 257,300 bacteria per gram with a range of 6100 to 350,000 bacteria per gram (table 1). The plain condensed skimmilk counts varied between 143,000 and 2,390,000. The gelatin contained 800 bacteria per gram.

*Methods of cleaning and sterilizing.* The washing of vats, sanitary piping, and freezers was done in an approved manner. The homogenizer was not taken apart. No steam was used for sterilizing but instead the entire system was flushed with a homemade calcium hypochlorite solution. The glass lined aging tank was sterilized by dashing the hypochlorite solution down the sides.

*Processing the mix.* The entire mix was pasteurized at a temperature of 165°F. for twenty minutes after which it was cooled to 120°F. for homogenizing. The mix was cooled with brine to 35°F. in the aging tank.

### *Results*

*Unsupervised mixes.* An examination of table 2, mixes 17 and 18, reveals that the average bacterial content of the mix was lower in this plant than in the others studied, probably due to the superior quality of milk and cream used. A pasteurizing efficiency of 99.53 and 99.88 per cent respectively reduced the bacterial counts to 2000 per gram. A temperature of 165°F. for twenty minutes would be expected to kill a larger percentage of the organisms than is ordinarily obtained at 145°F. for thirty minutes, but there seemed to be no practical advantage in such extreme pasteurization. In most instances equally as high efficiency has been obtained with 150°F. for thirty minutes (1). It is more costly to pasteurize at 165°F. and there is always some danger of a cooked flavor, although none was noticed in these trials.

Contamination from equipment was low, indicating that the homemade hypochlorite solution was fairly efficient as a sterilizing agent. The final counts on the two mixes were 47,600 and 11,100 bacteria per gram.

*Supervised mixes.* Plant V was the only plant studied where the unsupervised mixes were consistently lower in count than the supervised. It should be remembered that conditions as a whole were unusually good in this plant. A study of the methods indicated only two places for improvement; namely, taking the homogenizer apart to clean after use, and steaming equipment in place of using the hypochlorite solution as a sterilizing agent. In cleaning the homogenizer it was found necessary to use a knife to remove the accumulated "milk stone" from the valves.

An examination of the results obtained from mix 19[3] reveals a gradual increase in counts in each step of the process after pasteurization. At first this might appear to be due to contamination from the equipment. It is believed, however, that the increases are probably due to the development of thermophilic bacteria at the temperature of homogenizing (120°F.). This contention is supported by two facts: (*a*) the culture plates contained types of colonies not ordinarily encountered; and (*b*) the 400 per cent increase in counts between the "after pasteurizing" and the "before homogenizing" samples, under conditions which precluded the possibility of contamination. As previously explained, a period of about one hour elapsed between the time these two samples were taken. It would be unfair therefore to conclude from these results that the homemade hypochlorite is more efficient than steam as a sterilizing agent.

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[3] The supervisor was able to be present for only one mix.

### PLANT VI

Plant VI was a small plant with old equipment in poor condition and manned by poorly trained workmen. The boiler was in such poor condition that one and one-half hours time was required for heating the 1300 pound mixes to 145°F.

*Equipment.* The equipment consisted of a 200-gallon mixing vat, two similar aging vats, a tubular cooler in extremely bad condition, a rebuilt homogenizer, and two 40-quart horizontal brine freezers. Sanitary pipe and fittings were used.

*Raw materials.* The cream used averaged 19,807,000 bacteria per gram and was invariably slightly sour. The milk, freshly delivered averaged 3,410,000 bacteria per gram. The milk powder gave counts varying from 20,000 to 160,000 and the butter from 211,000 to 1,810,000. The gelatin contained 11,300 bacteria per gram.

*Methods of cleaning and sterilizing.* The vats and sanitary piping were washed with warm water (130°F.) and washing powder, and then rinsed with warm water but not steamed. The homogenizer was taken apart and washed but no attempt was made to sterilize it. The freezers were carelessly washed by rinsing with cold water, luke warm water and washing powder, and again rinsed with cold water, the head being removed and left off until the next freezing. Upon examination a scum was found around the fruit hopper seat.

*Processing the mix.* The entire mix consisting of milk, cream, butter, skimmilk powder, sugar, and gelatin was heated to 145°F. and the homogenizing immediately started. The temperature during homogenization varied from 145°F. at the beginning to 120°F. at the end. From the homogenizer the mix passed over the tubular cooler which reduced the temperature to about 80°F. The cooling was not started in the vat until the entire mix had been homogenized; the coil remaining stationary. Since it required one and one-half hours time to homogenize, a part of the mix was held at 80°F. in a poorly washed vat for about two hours. The mix was eventually cooled to 35°F. and frozen after six to ten hours aging.

### *Results*

*Unsupervised mixes.* As would be expected from the foregoing description of the methods, the pasteurizing efficiency was low (82.85 and 78.63 per cent in the two mixes) and gross contamination from equipment resulted in final counts of 680,000 and 580,000 bacteria per gram.

*Supervised mixes.* Between the unsupervised and supervised mixes, a new boiler was installed and the sanitary piping was rearranged so as to eliminate the cooler, which as mentioned above was in very poor condition.

The equipment was washed according to approved methods and steamed. Hot water at 180°F. was pumped through the homogenizer and since no steam connection was available for steaming the freezer, it was also scalded with water at 180°F.

With the new but inadequate boiler installed it still required fifty-five minutes to heat the mix to 145°F. The mix was held for thirty minutes before starting the homogenizer. The temperature was maintained above 140°F. during the entire homogenizing process. The mix ran directly into the aging vat and cooling was started as soon as the mix reached the coils.

Table 2, mixes 22 and 23, with final counts of 17,500 and 15,000, or an average of 16,200 bacteria per gram as compared to an average of 630,000 bacteria per gram in the unsupervised mixes, show practically no contamination from equipment.

## INGREDIENTS AS A SOURCE OF BACTERIA IN THE ICE CREAM MIX.

In all previous work on bacteria in ice cream the liquid milk products have been recognized as the chief source of bacteria in ice cream.

Table 5 shows clearly the source of the bacteria in the ice cream mixes studied in this work insofar as ingredients are concerned. The data here presented very forcibly emphasize the importance of the liquid milk products as the principal sources of bacteria in ice cream. Examination of the data shows that where cream and milk were used, they together, were responsible in most instances for over 99 per cent of the bacteria before pasteurization. This fact suggests the desirability of more effort on the part of the manufacturer toward securing milk and cream of better quality. In only one of the plants (plant V) was milk used which would pass the most liberal city ordinance for milk that was to be pasteurized.

The condensed milk in one case supplied 56 per cent of the total bacteria in the unpasteurized mix. In another case highly concentrated unsweetened condensed skimmilk (40 per cent solids) contained only 7600 bacteria per gram. Thus, while it made up 12.45 per cent of the total weight of the mix it was responsible for only 0.04 per cent of the total organisms before pasteurization (plant IV, mix 16). The milk used in this mix contained 3,490,000 bacteria per gram and supplied 99.92 per cent of the total bacteria. Butter was used in only a few mixes and in the majority accounted for less than 1 per cent of the bacteria present. Attention is called to the fact that gelatin has been a negligible factor as a source of bacteria in the ice creams studied. The same thing may be said of water and sugar.

In table 6 there is recorded the calculated number of bacteria added to each gram of mix by the various ingredients. It will be noted that cream added from 32,000 to nearly 12,000,000 bacteria to each gram of mix, with an average of almost 2,000,000. Milk added from 2300 to more than 34,000,000 and averaged a little over 7,000,000 per gram.

This table also indicates that condensed milk when used as the source of additional solids is a more potent source of bacteria in the mix than skimmilk powder when used for the same purpose. The condensed milk added from less than 1000 in one case to more than 2,000,000 in another case or an average of 377,668 bacteria per gram of mix. The skimmilk powder added from 1130 to 15,700 or an average of only 11,100 bacteria per gram of mix. In mix 23 the skimmilk powder added only 524 bacteria per gram but since only 34 pounds were used in a 1300 pound mix in contrast to 220 pounds of sweetened condensed milk in the same mix, it should be disregarded.

On first thought the above facts may seem of more importance than they really are. However, it should be remembered that condensed milk reduces to a considerable degree the bulk of the fluid milk needed in the mix. As shown in table 1 milk usually contains more bacteria per gram than the condensed milk. It is therefore evident that unless water is used in place of fluid milk in conjunction with milk powder the reduction in count due to using milk powder rather than condensed milk is of little consequence in the production of ice cream of low bacterial count. This is especially true where the entire mix is pasteurized. The condensed milk used was the product of three condenseries. The milk powder was obtained from three different sources. In eight mixes (5, 6, 7, 8, 16, 17, 18, 19) plain condensed milk was used while in four mixes (13, 14, 15, 23) sweetened condensed milk was used. The highest counts, viz., 11,800,000 and 15,400,000, were on sweetened condensed milk while the lowest, 7600, was on the heavy plain condensed milk. These results forcibly suggest the need of greater supervision of production on the part of the condenseries making bulk goods. The results also indicate considerable room for improvement in some milk powder factories.

The other ingredients butter, gelatin, water, and sugar were responsible for relatively few organisms.

## SUMMARY AND CONCLUSIONS

One of the objects of this investigation was to determine the possibility of producing a product of unquestioned sanitary quality under the most trying plant conditions, and to verify the results obtained in previous work. Table 4 emphasizes the results very well. The average count for all the unsupervised finished ice cream was 390,225 bacteria per gram, while the average for the supervised finished ice cream was only 39,127 bacteria per gram. If the results obtained in plant V are not averaged with the rest, the difference would be even more pronounced. The highest count of the finished product under supervision was 91,000 bacteria per gram while the lowest was 3200 bacteria per gram.

Only 5 of 12 unsupervised mixes were pasteurized as required by the Kansas law (mixes 1, 2, 14, 17, 18; table 2).

It is apparent from a study of tables 4, 5 and 6 that not enough effort is expended on the part of the manufacturers toward securing a more desirable quality of milk products.

The results included in this work verify in every detail the summary of the results of previous work (1).

From the data presented in this paper the following conclusions seem justifiable:

1. Cream and milk are the most important source of bacteria in the raw ice cream mix. In most instances these products supplied over 99 per cent of the total bacteria in the raw mix.
2. Condensed milk may, at times, be an important source of bacteria in the raw mix.
3. Gelatin, water, and sugar were negligible factors as sources of bacteria in the ice creams studied.
4. Thorough washing with an alkaline washing powder and sterilization of all equipment with live steam are essential factors in the production of ice cream of low bacterial content.
5. Carelessly washed tubular surface coolers are a considerable source of bacterial contamination in ice cream.
6. Conveying pipes, pumps, and homogenizers cannot be properly cleaned without being taken apart.
7. Hypochlorite solutions, when properly used, give satisfactory results in sterilizing ice cream plant equipment.
8. The use of pasteurizing equipment for two or more successive mixes without washing may result in high counts due to the growth of thermophilic bacteria.
9. The aging, without agitation, of improperly cooled mix (65°F.) in cans in a cold room (38°F.) results in a material increase in bacterial content in twenty-four hours.
10. Proper pasteurization is the most important factor governing the bacterial count of ice cream. Pasteurization at 145°F. for thirty minutes and homogenization at pasteurizing temperature results in counts of less than 100,000 bacteria per gram

in the finished ice cream, provided equipment contamination is reduced to a minimum. These results were obtained even with raw mixes containing as high as 34 million bacteria per gram.

11. If the bacterial flora of the mix is constituted of types easily destroyed by heat, high efficiency of pasteurization may be obtained in less time than is regularly prescribed.

12. Extreme pasteurizing temperatures (165°F.) are unnecessary in producing ice cream of low bacterial content.

13. Homogenization, even though contamination is negligible, usually results in an increased count, as determined by the plate method, probably due to breaking up of bacterial clumps.

14. Ice cream mix should be cooled as soon as possible after homogenizing, to prevent bacterial growth.

15. It is possible and practical to produce ice cream containing less than 100,000 bacteria per gram under all plant conditions, provided that efficient pasteurization is practiced, that the temperature is controlled during aging and that equipment is properly washed and sterilized. High bacterial counts indicate carelessness at some point in the manufacturing process which in turn indicates an undesirable, if not an unsafe, product. There can be no valid excuse for ice cream containing more than 100,000 bacteria per gram as determined by the plate method.

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| | |
|---|---|
| Adams, C. F. | Atherton Dairy, Atherton, Mo. |
| Adams, David P | Department of Agriculture, Nashville, Tenn. |
| Altweg, C. A | Carnation Milk Company, Oconomowoc, Wis. |
| Anderson, E. E | Extension Department, Corvallis, Ore. |
| Anthony, E. L | Morgantown, W. Va. |
| Arey, J. A | Dairy Extension, State College, Raleigh, N. C. |
| Astroth, Frank B | 1502 Raymond Avenue, St. Paul, Minn. |
| Atkeson, F. W | Dairy Department, Moscow, Idaho. |
| Baer, A. C | Dairy Department, Stillwater, Okla. |
| Beam, A. Leland | State College, Pa. |
| Bechdel, S. I | State College, Pa. |
| Becker, Raymond B | University Farm, St. Paul, Minn. |
| Beckman, H. C | 29 E. Madison Street, Chicago, Ill. |
| Bell, R. W | 1357 Park Road, Washington, D. C. |
| Besemer, A. M | In care of Golden State Milk Products Company, 425 Battery Street, San Francisco, Calif. |
| Brandt, P. M | Oregon Agricultural College, Corvallis, Ore. |
| Brannon, J. M | Dairy Department, Urbana, Ill. |
| Breed, Robert S | Geneva, N. Y. |
| Brew, James D | Dairy Department, Cornell University, Ithaca, N. Y. |
| Brandt, Carl A | 745 North Avenue, Bridgeport, Conn. |
| Brigham, Elbert S | St. Albans, Vt. |
| Brody, Samuel | Dairy Building, Columbia, Mo. |
| Brown, R. W | Agricultural College, Winnipeg, Canada. |
| Borland, A. A | Dairy Department, State College, Pa. |
| Bouska, F. W | 1526 S. State Street, Beatrice Creamery Company, Chicago, Ill. |

| | |
|---|---|
| Bush, M. G. | Cuba, N. Y. |
| Button, F. C. | Agricultural Experiment Station, Dairy Department, New Brunswick, N. J. |
| Burdick, Howland | Rhode Island State College, Kingsdon, R. I. |
| Bryan, K. V | Dairy Department, W. Lafayette, Ind. |
| Baltzer, A. C. | Dairy Department, E. Lansing, Mich. |
| Bennett, Frederick W | Dairy Department, Georgia State College of Agriculture, Athens, Ga. |
| Boyer, A. H. | New Haven Dairy Company, New Haven, Conn. |
| Bigelow, A. P. | Middlesex, Vt. |
| Boudewyns, Celestin | R. R. No. 1, Box 145, Westwood, N. J. |
| Boehr, J. W. | Dairy Department, Stillwater, Okla. |
| Britt, A. | City Consumers' Company, Paducah, Ky. |
| Bierman, Harlow | Experiment Station, College Park, Md. |
| Brown, Hamlin L. | Extension Dairyman, Gainsville, Fla. |
| Burke, A. D. | Dairy Department, Stillwater, Okla. |
| Bendixen, H. A. | Dairy Department, Moscow, Idaho. |

| | |
|---|---|
| Cade, L. S. | Climax Creamery, Shawnee, Okla. |
| Caine, George B | Logan, Utah. |
| Caldwell, R. E. | Blatchford Calf Meal Company, Waukegan, Ill. |
| Campbell, M. H. | Dairy Department, Urbana, Ill. |
| Campbell, Theodore A. | 38 Little Street, Matawan, N. J. |
| Cave, H. W. | Dairy Department, Manhattan, Kans. |
| Chumlea, Leon W. | Lebanon, Ind. |
| Clevenger, W. L. | Dairy Department, State College, Raleigh, N. C. |
| Clutter, J. G. | College Station, Tex. |
| Cohee, C. I. | 1211 Arch Street, Philadelphia, Pa. |
| Colman, H. N | Dairy Department, Corvallis, Ore. |
| Combs, W. B. | Dairy Division, University Farm, St. Paul, Minn. |
| Cook, Alfred | Plainsboro, N. J. |
| Cooledge, L. H. (deceased) | East Lansing, Mich. |
| Corbett, L. S. | Orono, Me. |
| Cunningham, O. C. | New Mexico College of Agriculture, Dairy Department, State College, N. M. |
| Camburn, O. M. | 4-A Oak Knoll, Arlington, Mass. |
| Cunningham, Walter S. | University of Arizona, Tucson, Ariz. |
| Cummings, Charles M. | Peterboro, N. H. |
| Clark, R. S. | Huntingdon County Farm Bureau, Huntingdon, Pa. |

| | |
|---|---|
| Dahlberg, A. C. | Experiment Station, Geneva, N. Y. |
| Dahlberg, A. O. | 175 Franklin Street, New York, N. Y. |

| | |
|---|---|
| Dahle, Chester D | Dairy Department, State College, Pa. |
| Darger, H. C | Blue Valley Creamery Company, Chicago, Ill. |
| Davis, H. P | Dairy Department, Lincoln, Nebr. |
| Darnell, A. L | College Station, Tex. |
| De Pew, H. F | State College, Durham, N. H. |
| Dice, J. R | Agricultural College, Fargo, N. D. |
| Doan, F. J | Dairy Department, College Park, Md. |
| Dunne, J. E | Hazelwood Ice Cream Company, East 6th and Main Streets, Portland, Ore. |
| Dvorachek, H. E | Fayetteville, Ark. |
| Drain, Harry D | Ohio State University, Columbus, Ohio. |

| | |
|---|---|
| Eckles, C. H | University Farm, St. Paul, Minn. |
| Ellenberger, H. B | University of Vermont, Burlington, Vt. |
| Ellington, E. V | Dairy Department, Pullman, Wash. |
| Epple, W. F | 311 Sylvia Street, W. Lafayette, Ind. |

| | |
|---|---|
| Fay, A. C | Department of Bacteriology, K. S. A. C. Manhattan, Kans. |
| Fisher, R. C | Storrs, Conn. |
| Fitch, J. B | Dairy Department, Manhattan, Kans. |
| Fisk, W. W | Cornell University, Ithaca, N. Y. |
| Frandsen, J. H | 1401 N. 33rd Street, Lincoln, Nebr. |
| Fraser, W. J | Urabana, Ill. |
| Fuller, J. M | New Hampshire College, Durham, N. H. |
| Fitts, E. B | Department of Agricultural Extension, State College, Pa. |
| Frevert, G. E | Newman, Calif. |
| Folger, Arthur | Davis, Calif. |
| Forbes, E. B | Institute of Nutrition, State College, Pa. |
| Fourt, D. L | University Extension Division, Boise, Idaho. |

| | |
|---|---|
| Gannon, E. A | Purdue University, Lafayette, Ind. |
| Gamble, J. A | University of Maryland, College Park, Md. |
| Gardner, M. H | Delavan, Wis. |
| Gearhart, C. R | Dairy Department, State College, Pa. |
| Gifford, Warren | Dairy Department, Morgantown, W. Va. |
| Goss, C. A | The Dairy Farmer, Des Moines, Iowa. |
| Goss, E. F | Iowa State College, Ames, Iowa. |
| Gregory, H. W | 416 Harvey Avenue, Lafayette, Ind. |
| Green, Sam H | 216 Pine Street, in care of California Dairy Council, San Francisco, Calif. |
| Grey, C. E | California Central Creamery Company, 417 Market Street, San Francisco, Calif. |
| Gullickson, T. W | University Farm, St. Paul, Minn. |
| Guthrie, E. S | Cornell University, Ithaca, N. Y. |

| | |
|---|---|
| Goodwin, O. T. | Michigan Producers Dairy Company, Adrian, Mich. |

| | |
|---|---|
| Hale, Joseph C. | Akron Pure Milk Company, Akron, Ohio. |
| Hammer, B. W. | Iowa State College, Ames, Iowa. |
| Hinman, G. M. | Dairy Inspector, 508 Peyton Building, Spokane, Wash. |
| Harding, H. A. | Box 834, Detroit, Mich. |
| Harmon, E. M. | Dairy Department, Columbia, Mo. |
| Harrison, Hartman K. | Linden Avenue and Dolphin Street, Baltimore, Md. |
| Hansen, A. P. | Director State Extension Creamery, Copenhagen, Denmark. |
| Harris, Roy T. | Madison, Wis. |
| Hayden, C. C. | Experiment Station, Wooster, Ohio. |
| Hastings, E. G. | University of Wisconsin, Madison, Wis. |
| Haslerud, E. J. | Dairy Department, Bozeman, Mont. |
| Harvey, Samuel H. | College Park, Md. |
| Heineman, H. E. O. | 327 S. LaSalle Street, Chicago, Ill. |
| Hening, J. C. | New York Experiment Station, Geneva, N. Y. |
| Herzer, F. H. | Agricultural College, Miss. |
| Hindley, R. W. | 1406 Carlisle Avenue, Racine, Wis. |
| Hiscock, Ira V. | 62 Park Street, New Haven, Conn. |
| Hooper, J. J. | Kentucky Agricultural College, Lexington, Ky. |
| Hooper, H. A. | Cornell University, Ithaca, N. Y. |
| Hoover, Jessie M. | Dairy Bureau, Washington, D. C. |
| Howell, A. J. | 619 E. Fourth, Ottawa, Kan. |
| Harper, W. Horace | Baltimore Dairy Council, 825 Fidelity Building, Baltimore, Md. |
| Huffman, Carl F. | Michigan Agricultural College, Dairy Department, E. Lansing, Mich. |
| Huggler, N. A. | In care of Carl Gluns, Clinton Street, Galeton, Pa. |
| Hunziker, O. F. | Blue Valley Creamery Company, Chicago, Ill. |
| Hutton, C. A. | Extension Division, Knoxville, Tenn. |
| Holdaway, C. W. | Blacksburg, Va. |
| Higgins, L. A. | Starkville, Miss. |
| Hazlewood, Ben P. | 1218 W. Clinch Avenue, Knoxville, Tenn. |

| | |
|---|---|
| Ingham, Leroy W. | College Park, Md. |

| | |
|---|---|
| Jackson, H. C. | 610 Superior Street, Grove City, Pa. |
| Jamison, Neal C. | Extension Division, Corvallis, Ore. |
| Jones, H. M. | Dairy Specialist, Brookings, S. D. |

Jones, Idwal R.................. Dairy Division, University Farm, St. Paul, Minn.
Jordan, H. C...................... 716 Clermont Street, Antigo, Wis.
Jewell, R. M...................... 618 East High Street, Mt. Vernon, Ohio.
Johnson, Robert E............... Storrs, Conn.
Judkins, H. F...................... Dairy Department, Amherst, Mass.

Keeney, M. H...................... Cedar Grove, N. J.
Kelley, Frank W................. Gridley Dairy Company, Milwaukee, Wis.
Kildee, H. H....................... Ames, Iowa.
Knapp, I. R........................ 60 West Main Street, Somerset, Pa.
Knox, Fredrick H................ Portland, Mich.
Koch, Arthur....................... Minerva, Ohio.
Koch, Irving O.................... Moore-Ross Milk Company, Columbus, Ohio.

Ladd, John E...................... Animal Husbandry Department, Rhode Island State College, Kingston, R. I.
LaMaster, J. P.................... Dairy Division, Clemson College, S. C.
Larson, C........................... State College, Brookings, S. D.
Larson, C. W...................... Dairy Bureau, U. S. D. A., Washington, D. C.
Latzer, R. L....................... Hudson, Mich.
Lehmkuhl, Henry................. 84 Exchange Street, Rochester, N. Y.
Lide, B. M., Jr.................... 2008 Pine Street, St. Louis, Mo.
Linn, J. W.......................... Dairy Department, Manhattan, Kans.
Little, R. E......................... International Milk Dealers' Association, 139 N. Clark Street, Chicago, Ill.
Lockwood, W. P. B.............. 58 Adella Avenue, West Newton, Mass.
Lucas, P. S......................... Dairy Department, E. Lansing, Mich.
Lush, R. H.......................... Dairy Department, Manhattan, Kan.

Macy, Harold...................... University Farm, St. Paul, Minn.
Magruder, Don G................ Extension Department, Washington State College, Pullman, Wash.
Manhart, V. O.................... Dairy Department, W. Lafayette, Ind.
Martin, G. L....................... 1043—18th Avenue S.E., Minneapolis, Minn.
Martin, Willard H.............. Dairy Department, Manhattan, Kan.
Mason, R. H....................... Dairy Department, Fayetteville, Ark.
Masurovsky, Benjamin........ Dairy Department, Lincoln, Nebr.
Mead, S. W......................... Davis, Calif.
Meigs, E. B......................... 1445 Rhode Island Avenue, Washington, D. C.
Meister, Albert................... Capitol Dairy, 315 Sixteenth Street, Sacramento, Calif.
Merrill, A. R...................... Storrs, Conn.
Monrad, K. J...................... Chris Hansen Laboratories, Little Falls, N. Y.

| | |
|---|---|
| Moore, J. S | Agricultural College, Miss. |
| Moseley, Thomas W | Huntley Experiment Farm, Huntley, Mont. |
| Morris, Arthur J | Cedar City, Utah. |
| Morton, George E | Fort Collins, Colo. |
| Mortensen, M | Iowa State College, Ames, Iowa. |
| Miller, Milton M | 442 East 58th Street, Portland, Ore. |
| Mudge, Courtland S | Davis, Calif. |
| Munn, M. D | 910 S. Mich. Avenue, Chicago, Ill. |
| Musser, Karl B | Peterboro, N. H. |
| Miyogishima, Masu | 263 Hatogoya Yogohata Mochi, The Fuji Dairy, Tokio, Japan. |
| Miyawaki, Atsushi | Professor of Dairying, Imperial University, Sappora, Japan. |

| | |
|---|---|
| McCarthy, Dennis A | Interstate Dairy Council, 1911–13 Arch Street, Philadelphia, Pa. |
| McCandlish, A. C | Claunch, Sorbie, Wigtownshire, Scotland. |
| McInerney, Thomas J | Dairy Department, Cornell University, Ithaca, N. Y. |
| McWhorter, G. E | Milledgeville, Ga. |
| Morrison, F. B | University of Wisconsin, Madison, Wis. |
| McDowell, J. C | Dairy Bureau, Washington, D. C. |

| | |
|---|---|
| Nafis, Louis F | 544 Washington Boulevard, Chicago, Ill. |
| Nelson, D. Horace | Dairy Division, University Farm, Davis, Calif. |
| Nelson, J. A | Montana State College, Bozeman, Mont. |
| Nevens, W. B | College of Agriculture, Urbana, Ill. |
| Newlander, J. A | 101 Brooks Avenue, Burlington, Vt. |
| North, Dr. C. E | 30 Church Street, New York, N. Y. |
| Norton, H. W | Lansing, Mich. |
| Nystrom, A. B | 1819 G Street, N.W., Washington, D. C. |

| | |
|---|---|
| Olson, N. E | Wichita Creamery Company, Wichita, Kans. |
| Olson, T. M | Dairy Department, Brookings, S. D. |

| | |
|---|---|
| Parker, H. N | Main and Orange Streets, Engineer Building, Jacksonville, Fla. |
| Parker, Joseph B | Dairy Bureau, Washington, D. C. |
| Pace, D. J | Maple Falls, Wash. |
| Parfitt, E. H | Purdue University, W. Lafayette, Ind. |
| Perry, E. J | New Brunswick, N. J. |
| Petersen, W. E | Dairy Department, University Farm, St. Paul, Minn. |
| Pearson, R. A | Ames, Iowa. |

| | |
|---|---|
| Pomeroy, Carl T | Health Officer, Montclair, N. J. |
| Potts, Roy C | Department of Agriculture, Washington, D. C. |
| Quigley, J. V | Consumers' League, 408 East 11th Street, Kansas City, Mo. |
| Rishoi, A. H | Dairy Department, Washington State College, Pullman, Wash. |
| Ragsdale, A. C | University of Missouri, Columbia, Mo. |
| Reed, O. E | Dairy Department, E. Lansing, Mich. |
| Regan, M. J | Dairy Department, Columbia, Mo. |
| Regan, W. M | Agricultural Experiment Station, Davis, Calif. |
| Renner, K. M | Dairy Department, Manhattan, Kans. |
| Roadhouse, C. L | University of California, Davis, Calif. |
| Robbers, Walter | Dairy Department, New Jersey Agricultural Station, New Brunswick, N. J. |
| Rogers, L. A | Dairy Bureau, Washington, D. C. |
| Ross, H. E | Cornell University, Ithaca, N. Y. |
| Ruehe, H. A | Dairy Department, Urbana, Ill. |
| Ruffner, R. H | State College, Raleigh, N. C. |
| Sheridan, Martin J., Jr | Blossom Hill Farms, Lebanon, N. J. |
| Sammis, J. L | Madison, Wis. |
| Scott, George W | Dairy Department, University of Alberta, Edmonton, Alberta, Canada. |
| Shepherd, J. B | Bureau of Animal Industry, Ardmore, S. D. |
| Sherman, J. M | Dairy Industry Building, Ithaca, N. Y. |
| Sidelmann, I. O | State College, Pa. |
| Slater, Edward K | 5th and Cherry Streets, Milwaukee, Wis. |
| Schaefer, O. G | University Farm, St. Paul, Minn. |
| Smith, R. W., Jr | Dairy Department, Massachusetts Agricultural College, Amherst, Mass. |
| Stocking, W. A., Jr | Dairy Department, Cornell University, Ithaca, N. Y. |
| Stoltz, R. B | Dairy Department, Columbus, Ohio. |
| Supplee, George C | Dry Milk Company Research Laboratory, Brainbridge, N. Y. |
| Stuart, Duncan | Bureau of Dairying, Washington, D. C. |
| Shutt, D. B | Division of Veterinary Science, Michigan Agricultural College, E. Lansing, Mich. |
| Sommer, H. H | Dairy Department, Wisconsin University, Madison, Wis. |
| Sutermeister, L. A | Dayton, Ohio. |
| Swett, W. W | Bureau of Dairying, Washington, D. C. |
| Thom, Charles | Bureau of Chemistry, Department of Agriculture, Washington, D. C. |

Thurston, L. M....................Dairy Department, University Farm, St. Paul, Minn.
Taylor, C. L......................1200 S. Clay Street, Louisville, Ky.
Troy, H. C........................Cornell University, Ithaca, N. Y.
Turner, Charles W...............Dairy Department, Columbia, Mo.
Tobias, James Henry..............136 State Street, W. Lafayette, Ind.

Urner, F. G.......................Urner-Barry Company, 173 Chambers Street, New York, N. Y.

Van Norman, H. E................National Dairy Association, 910 S. Michigan Avenue, Chicago, Ill.
Van Lange, Oscar................P. O. Box 3134, San Francisco, Calif.
Van Slyke, L. L...................Geneva, N. Y.

Wing, R. E........................Dairy Department, Connecticut Agricultural College, Storrs, Conn.
Walts, Charles C................Dairy Department, Storrs, Conn.
Washburn, R. M..................2239 Goodon Avenue, St. Paul, Minn.
Weaver, Earl.....................Ames, Iowa
Weld, Ivan C.....................Chestnut Farms Dairy, Washington, D. C.
White, G. C.......................Storrs, Conn.
White, William...................Dairy Division, Washington, D. C.
Wilbur, J. W......................Smith Hall, Purdue University, Lafayette, Ind.
Williams, G. A....................Purdue University, W. Lafayette, Ind.
Williams, V. M...................Animal Industry Division, State College Station, Raleigh, N. C.
Wing, H. H........................Cornell University, Ithaca, N. Y.
Winkjer, Joel G..................Dairy Division, Washingtn, D. C.
Wylie, C. Elmer..................Morrill Hall, University of Tennessee, Knoxville, Tenn.
Williams, P. S....................Dairy Department, State College, Pa.

Zeigler, P. R.....................Wright-Zeigler Company, Boston, Mass.

# CONSTITUTION AND BY-LAWS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

## (As revised 1925)

## CONSTITUTION

### NAME

*Article 1.* The name of the organization shall be the American Dairy Science Association.

### OBJECT

*Article 2.* The object of the Association shall be to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction, by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

### MEMBERSHIP

*Article 3.* Membership shall consist of two kinds (1) active, (2) associate.

*Article 4.* The qualifications for membership in the two classes are as follows: (*a*) Any person is eligible to active membership who is formally announced by an Agricultural College, or Experiment Station, or by the Bureau of Dairying of the United States Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or (*b*) anyone filling a position of responsibility connected with the dairy industry and who has had a college or university training in technical science, or anyone filling a responsible position in the industry of a professional character requiring a technical knowledge of dairying of a high order.

Any person is eligible to associate membership who is regularly enrolled in a collegiate course in a college of agriculture and who is specializing in dairying.

*Article 5.* Nominations for active membership shall be submitted to the Executive Committee in written form and signed by at least three members. Upon receiving the unanimous endorsement of the Executive Committee and paying the annual membership dues, the candidate shall be duly enrolled as a member of the Association.

*Article 5-a.* Associate membership is attained by election to membership in a local chapter of the American Dairy Science Association. Election may be by such means as are prescribed by the local chapter subject only to the qualification for associate membership as specified in Article 4, and the approval of the head of the Department of Dairying or Dairy Husbandry in the institution where the chapter is located. Associate membership expires two years after the holder ceases to be enrolled as a student.

### OFFICERS

*Article 6.* The officers of this Association shall be President and Vice-President, whose term of office shall be one year; a Secretary-Treasurer and a Journal Editor, whose term of office shall be two years. The term of office shall begin January first.

The President, the Vice-president and the Secretary-Treasurer and the presiding officers of the divisions of the Association shall constitute the Executive Committee.

*Article 6-a.* A representative of this Association to the National Research Council shall be elected for a term of three years. He shall be ineligible for reelection, shall be chosen from the members of the National Research Council Advisory Committee and shall be chairman ex-officio of the Committee. The term of office shall begin July first following his election.

### DUTIES OF OFFICERS AND MANNER OF ELECTION

*Article 7.* The duties of the officers shall be those usually pertaining to their respective offices. The Executive Committee shall pass upon all nominations for membership, all applications for divisions, sections, and chapters of the Association. It shall have the power to fix the time and place for the annual meeting and shall be authorized to transact such business of the Association as demands attention while the Association is not in session.

*Article 8.* On or before November first, the Secretary shall mail to each active member a blank on which he shall be entitled to express his choice for each office to be filled. This blank shall give the names of those serving in the offices to be filled for five years preceding and a report of the Committee on Elections which shall suggest the names of two members for each office to be filled. All ballots shall be counted by the Secretary and later verified by the President. In case no candidate has a majority by the first ballot, a second ballot shall be sent to the members, giving the names of the three leading candidates for each office and the number of votes received. The candidates receiving the most votes on the second ballot shall be declared elected. In case of tie on the second ballot, the decision shall be made by the Executive Committee.

### ORGANIZATION OF DIVISIONS AND SECTIONS

*Article 9.* Professional groups based on geographical considerations to be known as divisions of the Association and to be organized by active members of the Association may be authorized by the Executive Committee when such action shall seem expedient. The officers of the division shall be a chairman and such other officers as are provided by the division. The presiding officers of the divisions shall be ex-officio vice-presidents of the Association.

The divisions shall have the right to make by-laws for their own government which shall not be inconsistent with the constitution and by-laws of the Association.

Membership in division of the Association is open only to those regularly elected active members of the Association.

Any division may raise or collect funds to be expended for its own purpose.

*Article 10.* Professional groups based upon specialized interests to be known as sections of the Association and to be formed by not less than 10 active members may be authorized by the Executive Committee when considered for the best interests of the Association.

Such sections may elect their own officers and may make any rules for their own guidance not inconsistent with the constitution and by-laws of the Association.

*Article 10-a.* Local groups known as chapters may be authorized by the Executive Committee on petition from a group of members of the Association or of students qualified for associate membership according to Article 4. The officers of the chapter shall be a chairman and such other officers as the chapter shall provide. Membership is limited to those elected under conditions as given in Article 4. Each chapter may make rules for its guidance, not inconsistent with the constitution and by-laws of the American Dairy Science Association and may raise or collect funds to be expended for its own purposes.

### AMENDMENTS

*Article 11.* The constitution may be amended by a two-thirds vote at any regular meeting of the Association: provided the proposed amendments have been submitted to the Executive Committee in writing not less than thirty days previous to the meeting at which the vote is taken; and provided the proposed amendment is approved by a majority of the Executive Committee.

*Article 12.* The Executive Committee may at its discretion submit proposed amendments which have received the approval of the committee to the active members of the Association for vote by mail. An affirmative vote of two-thirds of all voting and which shall be not less than a majority of the active membership shall be necessary for approval.

## BY-LAWS

*Article 1.* The active membership dues shall be $5.00 a year, payable January first, each year.

*Article 1-a.* Annual dues for associate membership shall be fifty cents and shall be collected for the members of a chapter by such officer as the chapter may designate for the purpose and forwarded to the Secretary-Treasurer of the American Dairy Science Association.

*Article 1-b.* Associate members shall have the privilege of subscribing to the JOURNAL OF DAIRY SCIENCE at a rate of $2.50 a year, the subscription to begin with the calendar year and to continue twelve (12) months.

*Article 2.* The JOURNAL OF DAIRY SCIENCE shall not be sent to any member whose dues are not paid by April 11th of the year for which the membership is held.

*Article 3.* Any members in arrears for dues for more than one year shall thereby cancel membership but may be restored to membership without any action by the Association by payment of all arrears including dues for the current year.

*Article 4.* The time and place of the meetings of the Association shall be fixed by the Executive Committee.

*Article 5.* A quorum at any meeting for the transaction of business shall consist of not less than ten per cent of the active members.

*Article 6.* A committee of five on elections shall be appointed by the President. Said committee shall make a report at the annual meeting in which the names of two members shall be suggested for each office to be filled by mail ballot under the provision of the constitution. In addition thereto, a group of five or more active members may suggest other names for consideration but such requests shall be in the hands of the Secretary of the Association before November first.

*Article 7.* In conducting mail ballots only those ballots shall be counted which are received by the Secretary within four weeks from the date upon which ballots were mailed by the Secretary.

*Article 8. Section Meetings.* The time and place of meeting for the Sections of the Association shall be fixed by the Executive Committee. All other arrangements regarding the section meetings shall be made by the officers of the section.

*Article 9.* These by-laws may be amended at any regularly called meeting by a two-thirds vote of those present.

*Article 10.* The National Research Council Advisory Committee of this Association shall consist of six members in addition to the official representative of the Association. It is to be the function of this Committee to advise with the Association's representative to the National Research Council in the performance of his official duties. The members of the Committee shall be appointed by the President of the Association and shall serve for three years. The initial appointments shall be for such time as shall cause the terms of office of two members of the Committee to expire each year. The term of office shall begin July first.

# DAIRY NOTES

## PENNSYLVANIA

L. W. Morley, dairy extension specialist of the Pennsylvania State College has been selected by the American Jersey Cattle Club to head their extension and development division including Register of Merit work. Professor Morley is also chairman of the Extension Section of the American Dairy Science Association.

## KANSAS

N. E. Olson, associate professor of dairy husbandry in charge of dairy manufactures in the college and station resigned his position June 1st to go into commercial work at Wichita.

## MICHIGAN

L. H. Cooledge, research associate in bacteriology since 1912, died May 12, at the age of thirty-seven years. He was a native of South Dakota, graduating from the South Dakota College in 1911 and receiving the M.A. degree from the University of Missouri in 1912. Among his contributions to science was the development of the colorimetric hydrogen-ion method of determining the keeping qualities of dairy products. He was also among the first to study the occurrence of *Bacterium abortum* in the milk of the cow, and he has made numerous contributions on that subject.

## AMERICAN DRY MILK INSTITUTE

Dr. H. E. Van Norman has recently been elected president of the American Dry Milk Institute with headquarters at 160 North La Salle Street, Chicago. Dr. Van Norman has direct charge of the research and educational work of that association.

## BUREAU OF DAIRYING

J. H. McClain formerly in charge of the Bureau's extension work in the South has just been made head of the introduction section of the Bureau of Dairying. Mr. McClain fills the vacancy caused by the death a few weeks ago of Mr. S. C. Thompson who had been connected with the Bureau for a number of years.

## WESTERN DIVISION OF AMERICAN DAIRY SCIENCE ASSOCIATION, ANNUAL MEETING, MULTNOMAH HOTEL, PORTLAND, ORE., NOVEMBER 1 AND 2, 1925

*Tentative program*

November 1, 9:30 a.m.—General Assembly.
  Business Meeting.
  Reports of Committees.
November 1, 1:30 p.m.—Sectional Meetings.
  Production Section: Chairman, H. M. King, University of British Columbia, Secretary, E. E. Anderson, Oregon Agriculture College.
  Program: Speaker
  Discussion: Revision of Dairy Cattle Judging Contest Rules for 1926, J. E. Dorman, U. S. B. D. Salt Lake City, Chairman.
  Manufacturing Section: Chairman, G. D. Turnbow, University of California; Secretary, N. S. Golding, University of British Columbia.
  Program: Paper: "Developing Types of Cheese and Possibilities in the Cheese Industry," N. S. Golding.
  Discussion: Revision of Dairy Products Judging Contest Rules for 1926, H. R. Lochry, U. S. B. D., Salt Lake City, Chairman.
  Discussion on Prizes.
  Trial Judging of Ice Cream by Dairy Products Judging Coaches, G. D. Turnbow, University of California, in charge.
November 2, 9:30 a.m.—General Assembly.
  Address: "The Utilization of By-Products and its Influence on the Industry," Dr. L. A. Rogers, in charge Research Laboratories, Bureau of Dairying, Washington, D. C.
  Report of Committee on Standardization of Experimental Methods and Program of Problems, E. V. Ellington, Washington State College, Chairman.
  Suggestions from Dr. L. A. Rogers, U. S. Bureau of Dairying.
  Adjournment.

[President H. A. Bendixen, of the Western Division of the American Dairy Science Association, in sending this tentative program, says all indications are for a splendid meeting this year. All dairy workers particularly interested in western dairying should arrange to attend. Remember the dates November 1 and 2, and the place Portland Oregon.—*Editor.*]

# *Twentieth Annual Convention*

## AMERICAN DAIRY SCIENCE ASSOCIATION

*INDIANAPOLIS, INDIANA*
*OCTOBER 12 and 13*

❦

Hotel Severin will be headquarters for the A. D. S. A. Write for reservation mentioning the fact that you are a member of the association.

The meetings of the Association will be held at the Hotel Severin beginning at 9:00 a.m. Monday, October 12. Dean Alfred Vivian of Ohio State University will address the morning session. Many other prominent speakers will appear on the program.

The banquet will be held on Tuesday evening, October 13, at 6:30 p.m. A. J. Glover, editor of Hoards Dairyman, will be the principal speaker at the banquet. Results of the Students Judging Contest will be announced at this time. A program of interest to all members is promised.

*(Over)*

# SECTION II—MANUFACTURING

*Monday, October 12, 1925, 2 P.M.*

CALL TO ORDER BY CHAIRMAN,
H. F. Judkins, Amherst, Mass.

READING OF MINUTES, SECRETARY,
N. E. Olsen, Wichita, Kansas

REPORTS OF STANDING COMMITTEES,
- CARDS FOR THE NATIONAL CONTEST IN JUDGING DAIRY PRODUCTS, H. F. Judkins, Amherst, Mass.
- BACTERIOLOGICAL METHODS: A. MILK, B. ICE CREAM, R. S. Breed, Geneva, New York
- SCORE CARDS AND LEGAL STANDARDS, J. H. Frandsen, Lincoln, Nebraska
- ECONOMIC PHASES OF DAIRY MANUFACTURING, R. C. Potts, Bur. Agric. Economics, Washington, D. C.
- METHODS OF DETERMINING MILK SOLIDS IN FATS, O. R. Overman, Urbana, Illinois

REPORTS OF SPECIAL COMMITTEES,
- TESTING ICE CREAM FOR FAT AND SOLIDS, R. C. Fisher, Cincinnati, Ohio
- THE OPERATION OF COLLEGE CREAMERIES, P. M. Brandt, Corvallis, Oregon

SERIES OF 15 MINUTE TALKS,
- CAN A CITY MILK ORDINANCE BE ENFORCED? J. D. Brew, Ithaca, New York
- FEDERAL STATE BUTTER INSPECTION SERVICE AS A MEANS FOR QUALITY IMPROVEMENT, C. W. Fryhofer, St. Paul, Minn.
- CORRELATION OF THE SCORED BUTTER AND ITS METHOD OF MANUFACTURE WITH THE INFLUENCE OF YEASTS AND MOLDS, E. H. Parfitt, Urbana, Illinois
- JUDGING DAIRY PRODUCTS IN OUR HIGH SCHOOLS, C. L. Roadhouse, Davis, California
- RELATIONS BETWEEN THE COLLEGE DAIRY MANUFACTURES RESEARCH WORKER AND COMMERCIAL DAIRY INTERESTS, O. F. Hunziker, Chicago, Illinois
- THE EFFECT OF ICE CREAM INGREDIENTS UPON THE VISCOSITY OF THE MIX, P. A. Downs, Lincoln, Nebraska

# SOME FACTORS CONCERNING THE "PARTIAL NEUTRALIZATION" OF CREAM FOR BUTTERMAKING*

B. A. STIRITZ AND H. A. REUHE

*Department of Dairy Husbandry, University of Illinois, Urbana, Illinois*

Much of the butter made in creameries today is manufactured from cream in which the acid has been reduced by the addition of some weak alkali, as lime, soda ash or sodium bicarbonate. The purpose of neutralizing is to get the cream in shape for proper pasteurization. There is no doubt that the flavor is improved as well as the keeping quality where neutralization and pasteurization are used. Uniform butter can be made from day to day by controlling the acidity through neutralization and ripening of the cream. On the other hand, unless care is exercised, the use of a neutralizer may impair the quality of the butter by imparting a limy flavor, due to lime particles, or a soapy flavor because of the saponification of some of the fats.

It is not known just when the first neutralizer was used in the buttermaking process, but Flint, in 1858, stated that the use of soda and water will improve the quality of the butter (1). In 1901–1902 extensive experiments were carried out by Prof. G. L. McKay in which he used various alkalis for reducing the acidity of the cream (2) (3). As early as 1905, some creameries were using lime in buttermaking on a commercial scale (2). Since that time, the practice has spread rapidly. It is used practically in every creamery of the centralizer class and many of the smaller factories. From time to time, various alkalis were used for the reduction of the acid in cream (2) (4), but the general practice today is to use lime, soda ash or sodium bicarbonate or a combination of two of these.

In the use of the neutralizer, most plants use a 15 per cent mixture of lime and water (3) (4) or a 8 to 10 per cent solution of soda ash or sodium bicarbonate. To secure the best results in butter-

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* Received for publication May 9, 1925.

making, the cream must be pasteurized after it is neutralized, except when sweet cream is churned. In the latter, pasteurization is employed but the cream is not neutralized as the acid content is low.

The advantages gained from neutralizing cream are well known. It avoids excess fat losses and curd particles in the butter which results from the pasteurization of sour cream. To a certain extent, it prevents flavors which result from churning sour cream. Along with efficient pasteurization, it improves the keeping qualities of butter made from a high acid cream.

The object of these experiments was not to compare neutralized cream with unneutralized cream, but to compare the effect of the use of various neutralizers on the quality of the finished butter (made from the same cream), the keeping quality of the butter, and on the fat losses in the buttermilk.

Before the main experiments were carried out, many preliminary tests were run to determine the factors most favorable to the correct neutralization of the cream, such as the strength of the neutralizer to use; the time required for the neutralizer to react with the acid after it is added to the cream; and the amount of the reduction in acidity which takes place during the pasteurization process.

## EXPERIMENTAL WORK

In every case, the temperature of the cream, when neutralized, was between 90° and 100°F. This temperature was used, as the authors found (some unpublished results obtained during the summer of 1922 with lime) that lower temperatures tend to produce a butter with a neutralizer flavor; while 115°F. tends to give a scorched flavor to the butter. The temperature of 90° to 100°F. gave the best flavored butter.

In each pasteurization, the cream was heated to 160°F., held fifteen minutes, and then cooled as rapidly as possible.

### *Strength of the neutralizer*

The neutralizers used in the preliminary work were slaked lime, Lehigh Lime, soda ash and sodium bicarbonate. The Lehigh

Lime was used because it is a true representative example of relatively pure calcium lime as sold to creameries for neutralizing purposes. The strengths of the Lehigh Lime used were 10 15, and 25 per cent, those of the soda ash and sodium bicarbonate were 5, 10 and 20 per cent.

The lime mixtures were prepared as follows: for the 10 per cent Lehigh Lime mixture, 100 grams of lime were weighed and this amount was added to 900 cc. of hot water. For the 15 and 25 per cent mixtures, the same procedure was followed except that 150 and 250 grams were put in 850 and 750 cc. of hot water respectively.

In the case of the 10 per cent slaked lime mixture, 100 grams of rock lime were weighed, and to this, 500 cc. of boiling water were added. The lime was allowed to slake and then it was diluted with 400 cc. of water. In preparing the 15 and 25 per cent mixtures, the same procedure was followed. One hundred and fifty and 250 grams of rock lime were placed in separate jars. To the former, approximately 400 cc. of boiling water were added; to the latter, 300 cc. of boiling water. After the lime was slaked, each mixture was diluted with 450 cc. of water.

In using lime, an attempt was made to secure as fine a suspension of the lime particles as possible by shaking and by using hot water, so that the danger of large particles of lime showing up in the butter was minimized.

The soda ash and the sodium bicarbonate are both fairly soluble in water. In preparing the 5, 10, and 20 per cent solutions of these neutralizers, 50, 100 and 200 grams of each were weighed and placed in separate jars. To these, 950, 900 and 800 cc. of warm water were added in the order named.

Two sets of churnings were made with each strength neutralizer and the same cream was used to complete each set with the various strength neutralizers. The cream was divided into four equal lots of 2000 grams each. The acidity of each lot was determined as follows: A 17.6 cc. pipette was used to transfer 17.5 cc. of the cream to a white cup. The pipette was then rinsed with distilled water and the rinsings placed in the cup with the cream. Three to five drops of phenolphthalein solution were used as an indicator. The acid in the cream was then titrated with a tenth normal sodium hydroxide solution from a 50 cc. burette until a faint pink color appeared and remained for at least thirty seconds. The amount of the alkali used was then read from the burette and the total acidity in the cream was calculated as lactic acid.

One lot was neutralized with the stronger neutralizer; the second with the medium strength; the third with the weaker solution; and the fourth was not neutralized but was used as a check churning. In each case, except the last, the acidity was reduced to approximately the same point or near 0.2 per cent acid. In this series of experiments, the cream was not pasteurized. It was held in individual jars in a room at 35°F. for fifteen to eighteen hours. The cream was then warmed to 54°F., and churned in a glass dasher churn until the butter granules were about the size of wheat grains. The time of churning was noted in each case. The buttermilk was drained through two thicknesses of cheese cloth to prevent the larger butter granules from getting into the buttermilk. A sample was saved in each case. Each lot of butter was then washed with ½ gallon of water at 58°F. No salt was added. The butter was then worked with wooden ladles and placed in storage at 35°F. The next day these lots of butter were scored mainly for quality because the main object was to see the effect of the various strength neutralizers on the flavor of the butter.

The buttermilk was tested by the normal butyl alcohol method. Tracy and Overman (5) found this test checked very close with the Roese-Gottlieb method of the determination of fat. This test is as follows: A double neck skimmilk bottle, as used in the Babcock Test, was used. Two cubic centimeters of normal butyl alcohol were pipetted into each bottle. Eight and eight-tenths cubic centimeters of a well mixed sample of buttermilk were placed in each bottle from a 8.8 cc. pipette. Nine cubic centimeters of sulphuric acid (specific gravity 1.82 to 1.83) were added and mixed with the milk by rotating the bottle. When the color of the mixture had turned to a dark brown, the bottle was centrifuged for five minutes. Then warm distilled water was added up to the base of the neck of the bottle; the bottle was centrifuged two minutes; warm water was added until the fat column rose in the capillary neck of the bottle; the bottle was centrifuged one minute; and then the fat column was read with the aid of a pair of dividers. The reading which was obtained was multiplied by two as a 9 gram sample of buttermilk was used in an 18 gram test bottle.

The results which were obtained are found in table 1.

### *Time for the neutralizer to react with the acid in the cream*

To determine the time which is necessary for the complete reaction of the neutralizer upon the acid in the cream, the following experiments were carried out. The neutralizers used were: 5 per cent soda ash; 5 per cent sodium bicarbonate; 10 per cent Lehigh Lime; 10 per cent slaked lime; 10 per cent Peerless Lime; 10 per cent Milk of Magnesia (Allwood); 10 per cent Kelly Island Lime, a mixture made by mixing equal parts of a 5 per cent soda ash solution and a 10 per cent Lehigh Lime mixture before adding it to the cream; and a mixture of equal parts of a 5 per cent sodium bicarbonate solution and a 10 per cent Lehigh Lime mixture before adding it to the cream. The method used in preparing the slaked lime, Lehigh Lime, soda ash and sodium bicarbonate has been outlined. The other limes were prepared in the same way as was the Lehigh Lime.

The following chemical analyses of the various neutralizers were either furnished by the manufacturers or made by the Chemistry Department, University of Illinois.

The soda ash was "C.P." sodium carbonate crystals and the sodium bicarbonate was "C.P." crystals as manufactured and sold by Powers-Weightman-Rosengarten Company of Philadelphia.

The slaked lime was prepared from pure calcium oxide with the following maximum limit of impurities:

| | *per cent* |
|---|---|
| Volatile substances | 7.500 |
| Chlorides | 0.005 |
| Silica | 0.100 |
| Sulphates | 0.200 |
| Alumina | 0.350 |

The Lehigh Lime contains:

| | per cent |
|---|---|
| Calcium hydrate | 96.90 |
| As listed: | |
| Calcium oxide | 72.93 |
| Loss (moisture) | 23.97 |
| Iron and alumina | 0.48 |
| Silica | 1.16 |
| Magnesium oxide | 1.25 |

The Peerless Neutralizer Lime contains:

| | per cent |
|---|---|
| Calcium hydroxide | 97.35 |
| Calcium carbonate | 1.42 |
| Iron and alumina | 0.37 |
| Silica | 0.52 |
| Magnesium oxide | 0.25 |
| Sulphates | trace |

The Kelly Island Lime contains:

| | per cent |
|---|---|
| Calcium oxide | 49.70 |
| Magnesium oxide | 32.60 |
| Silica | 0.30 |
| Iron and alumina | 0.20 |
| Loss on ignition | 17.00 |

The Milk of Magnesia (Allwood Laboratories) Lime contains:

| | per cent |
|---|---|
| Insoluble sulphur dioxide | 0.14 |
| Iron oxide | 0.38 |
| Aluminum oxide | 0.04 |
| Calcium oxide | 62.94 |
| Magnesium oxide | 27.24 |
| Loss on ignition | 8.64 |

Six deteminations were made with each neutralizer. One thousand grams of cream were placed in the inner vessel of a double boiler. The outer vessel was filled with water and heated over a gas flame. When the temperature of the cream reached 100°F. the inner vessel containing the cream was removed and placed in a water bath which had a temperature of 100°F. The acidity was determined with tenth normal sodium hydroxide and calculated as lactic acid. The neutralizer was added to the cream during the thorough agitation with a large spoon. The acidity was determined after one minute, two minutes, four minutes, five minutes, ten minutes, fifteen minutes, twenty minutes and twenty-five minutes. The results which were obtained are found in table 2.

### *Reduction in acidity due to the pasteurization process*

Every creameryman has experienced some reduction in the acidity of cream during the pasteurization process. Experiments were made to determine the reduction of acidity during pasteurization. All neutralizers used in the preceding set of experiments were used.

Each experiment was carried on as follows: One thousand grams of cream were placed in the inner vessel of a double boiler and neutralized at 100°F. to approximately 0.2 per cent acid. The cream was held at this temperature long enough for the complete reaction of the alkali and acid. Fifteen minutes after the neutralizer was added, the amount of acidity was determined with tenth normal sodium hydroxide, the acidity being calculated as lactic acid. The cream was then heated to 160°F. in the double boiler, held at this temperature for fifteen minutes and then cooled with water to approximately 100°F. The acidity was again determined and the difference in acidities, before and after pasteurization, was taken as the reduction due to pasteurization. The results are shown in table 3.

### *Effect of various neutralizers on the quality of the butter, storage properties and fat losses in the buttermilk*

After the preliminary tests to determine the best strength of each neutralizer to use and also the amount of the reduction in acidity to expect during the pasteurization process, larger churnings were made to determine the effects of the various neutralizers on the quality of the butter and on the fat losses in the buttermilk. The neutralizers which were used were 5 per cent solutions of soda ash and sodium bicarbonate and 10 per cent mixtures of the following limes: slaked lime; Lehigh Lime; Milk of Magnesia (Allwood); Kelly Island Lime and Peerless Lime. These were used alone and also in various combinations.

Each of these churnings was made with 50 pounds of cream. The churns used were Victor Juniors, number one, with two roll internal workers, each having a capacity of 30 pounds of finished butter. Both churns were operated from the same line shaft and had the same speed.

The cream used was of average quality as received at a large commercial plant. Sufficient cream was taken for all churnings in each series. The cream was placed in a 30 gallon, double jacketed starter can and the temperature was raised to 100°F. A sample was taken and tested for butterfat by the ordinary Babcock test for cream. The acidity was determined as lactic acid with a tenth normal alkali solution.

Lots of cream weighing exactly 50 pounds were placed in 10 gallon milk cans. These cans were placed in a large water tank and surrounded by water having a temperature of 100°F. Then each lot was neutralized with a specific neutralizer to approximately 0.20 per cent acid. The cream was agitated thoroughly with a stirring rod as the neutralizer was added. Fifteen minutes after the addition of the neutralizer, the acidity was again determined. If it was not at approximately 0.2 per cent acid, a sufficient amount of the neutralizer was added to lower the acidity to the desired amount. The water surrounding the 10 gallon milk cans was then heated to about 180°F. by using live steam. The cream in each can was stirred occasionally with a metal stirring rod and the temperature was watched carefully. When the temperature of the cream reached 160°F., the water which surrounded the cans was cooled to the same degree. The cream was allowed to remain at this temperature for fifteen minutes. The hot water was drawn off and the cans surrounded with water at approximately 68°F. (temperature of the tap water in the creamery). The cream was allowed to remain in this water with occasional stirring until the temperature was lowered to near 75°F. No starter was used. Each lot of cream was then placed in a cold room (35°F.) and held for twelve to fifteen hours. After this period of holding, two cans were removed at a time placed in warm water, and the cream heated to 54°F., the churning temperature in each case. It was held at this temperature for thirty minutes previous to churning. Each lot was tested for acidity and poured through a strainer into a Victor Junior, number one churn. No color was added. The cream was churned until the butter granules were the size of wheat grains. The exact time of churning was taken. The butttemilk was drained off and a sample taken for a fat test. The butter was washed by adding 50 pounds of water and revolving the churn in high gear for ten revolutions. In each case, the temperature of the wash water was the same as that of the buttermilk when it was drawn from the churn. The wash water was drawn off and the butter was salted at the rate of two-thirds ounce of salt to each pound of butterfat in the cream as calculated from the weight and test of the cream. The dry salt method was used. The butter was worked by revolving the churn in low gear with the workers in operation for twenty-four revolutions. Four 1 pound samples were taken from each churning and placed in the storage room at a temperature of 35°F. One sample was scored the next day and the other three, at intervals of thirty days (thirty, sixty and ninety days).

The sample of buttermilk from each churning was tested by the normal butyl alcohol method, as previously outlined.

Each lot of cream in a series of churnings was handled exactly the same in every respect. They were weighed, neutralized, pasteurized, cooled and held as a unit. Two lots were warmed to the churning temperature and churned at the same time in similar churns which were driven by the same line shaft and motor.

The cream was stirred thoroughly as it was neutralized, heated and cooled but care was exercised not to cause mealiness in the finished butter by too violent agitation. In each case, the neutralizer solution was shaken well before it was used. When lime solutions were used, the mixture was allowed to stand for a few seconds after being mixed to allow any large particles to settle to the bottom before taking any out for use. This would tend to eliminate a limy flavor in the butter due to the presence of lime particles.

The results which were obtained with the various churnings are found in tables 1 to 6.

## DISCUSSION OF RESULTS

### *Various strengths of neutralizers and the quality of the butter*

The effect of the various strengths of neutralizers on the quality of the finished butter is found in table 1. The general practice in creameries is to use an 8 or 10 per cent solution of soda ash or sodium bicarbonate and a 15 per cent suspension of lime in water. The latter is made usually by adding 85 pounds of hot or boiling water to 15 pounds of the lime or in this proportion if a larger amount is desired. The results show clearly that the better quality of butter results from using the weaker solutions. In each case, the cream which was neutralized with the weaker solutions gave a bettter quality of butter than the check or unneutralized churning. This cannot be said of the churnings which were neutralized with the stronger solutions because of the decided neutralizer or soapy flavor which resulted in the finished butter.

In considering the scores of the butter from each churning, the 5 per cent solution of soda ash or sodium bicarbonate resulted in a finished quality of 1.5 to 4 per cent better than did the 10 per cent solution, while it was 8 to 9 per cent better than the 20 per cent solution. Practically the same thing can be said when using lime. The 10 per cent suspension gave a finished quality of 1 to 2 per cent better than the 15 per cent mixture and 2 to 9 per cent better than the 25 per cent mixture.

### *Various strengths of neutralizers and the loss of fat in the buttermilk*

Table 1 shows the effect of the various strengths of neutralizers on the loss of the butterfat in the buttermilk. The weaker strength of each neutralizer resulted in the lower fat losses. This is true in each case except the sodium bicarbonate. This exception gave practically no difference between the 5 and 10 per cent solutions. The stronger solutions resulted in the per cent of butterfat in the buttermilk increasing from 0.60 and 0.79 to 0.81 and 0.94 per cent. The soluble neutralizers do not act the same as do the limes. The addition of the weaker lime mixtures resulted in a lower fat loss as compared with the check or unneutralized churning; while the soluble neutralizers resulted in a higher far loss (even with the weaker solutions) than did the check churning.

The results given in table 1 show that the use of the weaker neutralizers had two advantages; a better quality butter, and a more complete churning. Both of these factors are vital in the operation of a creamery.

TABLE 1

*Effect of the various strength neutralizers on the quality of the butter and on the butterfat lost in the buttermilk*

| NEUTRALIZER USED | STRENGTH | RELATIVE SCORE* | PER CENT OF FAT IN BUTTERMILK |
|---|---|---|---|
| | *per cent* | | *per cent* |
| Soda ash | Check | 84 | 0.62 |
| | 5 | 100 | 0.65 |
| | 10 | 96 | 0.74 |
| | 20 | 92 | 0.87 |
| Sodium bicarbonate | Check | 90 | 0.58 |
| | 5 | 100 | 0.79 |
| | 10 | 99 | 0.77 |
| | 20 | 91 | 0.94 |
| Lehigh lime | Check | 95 | 0.82 |
| | 10 | 100 | 0.60 |
| | 15 | 98 | 0.73 |
| | 25 | 91 | 0.81 |
| Slaked lime | Check | 93 | 0.74 |
| | 10 | 100 | 0.67 |
| | 15 | 99 | 0.74 |
| | 25 | 98 | 0.90 |

* The relative score is given on a percentage basis. The best butter was given a factor of 100; the others as percentages of the best. Each figure represents the average of two or three churnings.

### *The reaction of the neutralizer and the acid which is present in the cream*

Table 2 shows the rapidity with which the various neutralizers react with the acid in the cream and the time which must lapse to insure a complete reaction. To discuss properly the data and that which follows, it is well to group the various neutralizers into different classes, since some of them act practically the same. As the soda ash and the sodium bicarbonate are soluble in water, they will be called the soluble neutralizers. The Lehigh Lime, the Peerless Lime and the slaked lime are composed mainly of calcium hydroxide and these will be known as the calcium limes. The Milk of Magnesia (Allwood) Lime and the Kelly Island Lime contains much more magnesium hydrate than the calcium limes so they will be known as the magnesium limes. The other group will be known as the mixed neutralizers because they are composed of equal parts of a10 per cent solution of Lehigh Lime and a 5 per cent solution of either soda ash or sodium bicarbonate.

TABLE 2*

*The rapidity with which the various neutralizers react with the cream prior to pasteurization*

| NEUTRALIZER USED | PER CENT ACIDITY AT VARIOUS TIMES | | | | | | | | | TIME FOR COMPLETE REDUCTION TO TAKE PLACE |
|---|---|---|---|---|---|---|---|---|---|---|
| | Before Adding | 1 minute | 2 minutes | 4 minutes | 5 minutes | 10 minutes | 15 minutes | 20 minutes | 25 minutes | |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *minutes* |
| Lehigh Lime | 0.62 | 0.38 | 0.35 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 4 |
| Slaked lime | 0.63 | 0.30 | 0.24 | 0.20 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 5 |
| Peerless Lime | 0.63 | 0.36 | 0.30 | 0.20 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 5 |
| Allwood Lime | 0.60 | 0.33 | 0.28 | 0.22 | 0.19 | 0.18 | 0.15 | 0.15 | 0.15 | 15 |
| Kelly Island Lime | 0.58 | 0.35 | 0.26 | 0.22 | 0.19 | 0.17 | 0.17 | 0.17 | 0.17 | 10 |
| Soda ash | 0.71 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 1 |
| Sodium bicarbonate | 0.64 | 0.32 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 2 |
| 50–50 mixture Lehigh Lime and soda ash | 0.62 | 0.30 | 0.25 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 4 |
| 50–50 mixture Lehigh Lime and sodium bicarbonate | 0.64 | 0.32 | 0.29 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 4 |
| Average for all | | | | | | | | | | 4.5 |

* The table gives the averages of six determinations for each neutralizer.

The soluble neutralizers react almost immediately with the acid in the cream. If the neutralizer and the cream are agitated well at the time of mixing, it is safe to check the acidity of the cream in four minutes (this allows a safety factor of two minutes) and thus be certain that there will be no further reduction in the acidity prior to pasteurization.

All the lime neutralizers react slower in reducing the acidity because they are not completely soluble. No two classes of lime neutralizers react exactly alike. The calcium limes react completely in four to five minutes. If the cream is agitated thoroughly as the calcium limes are added, one can be sure that the reaction is complete in seven minutes (which includes two minutes as a safety factor) and there will be no further decrease in the acidity of the cream before pasteurization.

The magnesium limes react slower than do the calcium limes. This can be explained by the fact that magnesium hydroxide is much less soluble than calcium hydroxide. It is well to wait seventeen minutes after adding the magnesium lime before checking the acidity of the cream. This allows a safety factor of two minutes to insure a complete reaction.

The data in table 2 show the rapidity of the reaction for each neutralizer. The calcium limes react rapidly the first minute and then slower until the complete reaction takes place in four or five minutes. The magnesium limes react with practically the same speed as the calcium limes for the first five minutes and then much slower until the reaction is completed in ten to fifteen minutes. This slowing down is due probably to the complete reaction of the calcium hydroxide after five minutes which leaves the less soluble magnesium hydroxide to react with the acid in the cream.

The mixed neutralizers react very similar to the calcium limes. Six minutes should elapse between the addition of the neutralizer to the cream and the time when the acidity is again determined. It will be noted that the acid reduction is very rapid at first because the soluble neutralizer present reacts immediately, but it takes time for the reaction between the lime and the acid.

The time required for a complete reaction between the neutralizer and the acid in the cream (provided the two are agitated thoroughly) is found in table 2. The average time for all of the neutralizers to react is five and one-half minutes. All of the classes of neutralizers complete the reaction in five minutes or less except the magnesium limes; these require ten to fifteen minutes.

### *The reduction in acidity during the pasteurization process*

The reduction in acidity which takes place during the pasteurization process is given for each neutralizer in table 3. During this process as well as previous to it, the neutralizers in each group act similarly.

TABLE 3*

*Reduction in acidity during the pasteurization process*

| NEUTRALIZER USED | TIME HELD AFTER NEUTRALIZATION AND BEFORE PASTEURIZATION | REDUCTION DURING PASTEURIZATION IN THE PER CENT ACIDITY |
|---|---|---|
| | *minutes* | *per cent* |
| Lehigh Lime | 15 | 0.018 |
| Slaked lime | 15 | 0.030 |
| Allwood Lime | 15 | 0.050 |
| Peerless Lime | 15 | 0.040 |
| Kelly Island Lime | 15 | 0.058 |
| Soda ash | 15 | 0.068 |
| Sodium bicarbonate | 15 | 0.075 |
| 50-50 Mixture Lehigh Lime and soda ash | 15 | 0.027 |
| 50-50 mixture Lehigh Lime and sodium bicarbonate | 15 | 0.047 |
| Average for all | | 0.046 |

* This table gives the average of six determinations for each neutralizer.

Most creamery operators depend on some reduction in acidity during pasteurization. Opinions vary as to the amount of this reduction. Some claim that a reduction of only 0.05 per cent is obtained; others state that there is at least a reduction of 0.1 per cent. As a matter of fact, this will be governed by several factors: first, by the neutralizer used; second, whether or not the time allowed between the addition of the neutralizer and the checking of the acidity is long enough; third, the thoroughness of mixing the neutralizer and the cream; and last, the care with which the neutralizing solution is prepared. If the cream and neutralizer are not mixed thoroughly, the reduction in acidity may not be complete in the time allowed for the reaction, and it will be completed during pasteurization. Thus a greater decrease is shown during pasteurization than would have been the case if the neutralizer and cream had been mixed properly. If the neutralizing solution is not properly prepared and the particles of lime are large, it will take longer for a complete reaction between the lime and the acid in the cream. A fine suspension of lime is most desirable.

The reaction of the calcium limes was complete before pasteurization, since the data show a reduction in acidity during pasteurization of only 0.02 to 0.03 per cent. This reduction may have been due, not so much to completing the action of the lime on the acid, as to the driving off of volatile substances such as carbon dioxide.

The reduction when the magnesium limes were used varied between 0.05 and 0.055 per cent. Possibly, the large reduction was due to the fact that the reaction between the lime and the acid which was brought about more completely by the increased heat. It may have been partially due, also, to the driving off of volatile substances.

The soluble neutralizers gave the largest reduction during pasteurization. It varies between 0.065 and 0.075 per cent. When these neutralizers were added to cream, carbon dioxide was liberated, and in sufficient quantities to cause the cream to foam. The foaminess was more pronounced with sodium bicarbonate than it was with soda ash. Carbon dioxide is soluble in water but rather unstable and is quickly driven off by heat. This accounts for the reduction during pasteurization rather than a further reaction between the neutralizer and the acid in the cream.

The mixed neutralizers reacted as sort of averages between calcium limes and the soluble class. Such reactions might be expected as they contained equal parts of both Lehigh Lime and the soluble neutralizers. There is more reduction than with lime alone and less than with the soluble class. The reduction varies from 0.025 and 0.047 per cent acidity.

The average reduction for all neutralizers was 0.045 per cent.

The calcium limes and the mixed limes gave a reduction less than the average: the soluble neutralizers and the magnesium limes more than the average.

The reduction during the pasteurization process in commercial practice coincides with the work reported above and which was carried out on a small experimental scale. The following figures bear out this evidence. Five equal lots of the same cream were neutralized with Lehigh Lime, Peerless Lime, Slaked Lime, Kelly Island Lime and Allwood Lime. They were pasteurized at 160°F. for fifteen minutes. The acidities prior to pasteurization and after the cream had been held at 100°F. for fifteen minutes with the added neutralizer were: 0.20, 0.21, 0.19, 0.20, and 0.19 per cent, respectively. The acidities after pasteurization were: 0.17, 0.18, 0.16, 0.16, and 0.15 per cent. The reductions were: 0.03, 0.03, and 0.03, 0.04, and 0.04 per cent, respectively. These figures verify the results obtained in the smaller experiments. (The first three are calcium limes and the other two are magnesium limes.)

### *The relation of the various neutralizers to the butterfat lost in the buttermilk*

Tables 4 and 5 gives the results of the various neutralizers on the butterfat lost in the buttermilk. The neutralizers are grouped as individual neutralizers, similar neutralizers, and in the various combinations.

Whenever soda ash was used, either by itself or with Lehigh Lime, the test of buttermilk was the highest. In table 4, the average for all individual neutralizers was 0.701 per cent butterfat. The soda ash gave results from 0.086 to 0.134 per cent above this average. This is to be expected for soda ash tends to saponify the fat and also stabilize an emulsion. Either of these factors would account for a higher fat loss in the buttermilk.

Sodium bicarbonate did not give the same results as soda ash. The fat losses were below the average for all individual neutralizers except in one case. When Lehigh Lime and sodium bicarbonate were mixed before they were added to the cream the fat loss was greater than when they were used separately; yet it was only slightly above the average for all or 0.011 per cent for five determinations. Excess fat losses did not result from the use of sodium bicarbonate as was the case when soda ash was used.

TABLE 4

*The relation of the various neutralizers to the butterfat lost in the buttermilk and the time to churn*

| NUMBER OF CHURNINGS | NEUTRALIZER | ACIDITY OF THE CREAM AT START | ACIDITY OF THE CREAM TO CHURN | TEST OF THE BUTTERMILK | TEST OF THE BUTTERMILK ABOVE OR BELOW THE AVERAGE FOR ALL CHURNINGS | TIME TO CHURN IN MINUTES |
|---|---|---|---|---|---|---|
| 33 | Average of all combined..... | 0.54 | 0.22 | 0.701 | 0.000 | 16.0 |
| | The individual neutralizers | | | | | |
| 2 | Kelly Island Lime | 0.54 | 0.22 | 0.620 | −0.081 | 16.5 |
| 2 | Slaked lime | 0.54 | 0.21 | 0.650 | −0.051 | 15.5 |
| 2 | Allwood Lime | 0.54 | 0.22 | 0.595 | −0.106 | 15.5 |
| 2 | Peerless Lime | 0.54 | 0.225 | 0.580 | −0.121 | 16.0 |
| 5 | Lehigh Lime | 0.50 | 0.24 | 0.638 | −0.063 | 17.2 |
| 3 | Soda ash | 0.47 | 0.21 | 0.787 | +0.086 | 16.5 |
| 3 | Sodium bicarbonate | 0.47 | 0.21 | 0.680 | −0.021 | 17.5 |
| 5 | 50–50 mixture Lehigh Lime and soda ash | 0.55 | 0.22 | 0.794 | +0.093 | 16.1 |
| 5 | 50–50 mixture Lehigh Lime and sodium bicarbonate | 0.55 | 0.22 | 0.712 | +0.011 | 16.2 |
| 2 | Lehigh Lime to 0.4 per cent and soda ash to 0.2 per cent acidity | 0.65 | 0.20 | 0.835 | +0.134 | 15.0 |
| 2 | Lehigh Lime to 0.4 per cent and sodium bicarbonate to 0.2 per cent acidity | 0.65 | 0.20 | 0.630 | −0.071 | 14.5 |
| | Similar neutralizers | | | | | |
| 6 | Soluble | 0.47 | 0.21 | 0.733 | +0.032 | 17.0 |
| 14 | Mixed | 0.58 | 0.215 | 0.748 | +0.047 | 15.1 |
| 9 | Calcium limes | 0.52 | 0.22 | 0.649 | −0.052 | 16.6 |
| 4 | Magnesium limes | 0.55 | 0.22 | 0.607 | −0.094 | 15.7 |

The use of soda ash resulted in the highest fat losses, the sodium bicarbonate was about the average and the other extreme was obtained when limes were used. In each case, the limes were

TABLE 5

*The relation of the various neutralizers to the butterfat lost in the buttermilk and the time to churn*

Various combinations

| NUMBER OF CHURNINGS | NEUTRALIZER | ACIDITY OF THE CREAM AT START | ACIDITY OF THE CREAM TO CHURN | TEST OF THE BUTTERMILK | TEST OF THE BUTTERMILK ABOVE OR BELOW THE AVERAGE FOR ALL CHURNINGS | TIME TO CHURN *minutes* |
|---|---|---|---|---|---|---|
| 2 | Kelly Island Lime | 0.54 | 0.22 | 0.620 | −0.110 | 16.5 |
| 2 | Lehigh Lime | 0.54 | 0.23 | 0.615 | −0.115 | 16.5 |
| 2 | Allwood Lime | 0.54 | 0.22 | 0.595 | −0.135 | 15.0 |
| 2 | Peerless Lime | 0.54 | 0.225 | 0.580 | −0.150 | 16.0 |
| 2 | Slaked lime | 0.54 | 0.21 | 0.650 | −0.080 | 15.5 |
| 2 | 50–50 mixture Lehigh Lime and soda ash | 0.475 | 0.22 | 0.785 | +0.055 | 16.0 |
| 2 | 50–50 mixture Lehigh Lime and sodium bicarbonate | 0.475 | 0.215 | 0.840 | +0.110 | 16.5 |
| 2 | Lehigh Lime | 0.475 | 0.21 | 0.730 | 0.000 | 18.5 |
| 2 | Soda ash | 0.475 | 0.20 | 0.890 | +0.160 | 17.5 |
| 2 | Sodium bicarbonate | 0.475 | 0.215 | 0.760 | +0.030 | 18.5 |
| 2 | Lehigh Lime | 0.54 | 0.23 | 0.615 | −0.115 | 16.5 |
| 2 | Peerless Lime | 0.54 | 0.225 | 0.580 | −0.150 | 16.0 |
| 2 | Slaked lime | 0.54 | 0.21 | 0.650 | −0.080 | 15.5 |
| 3 | Lehigh Lime | 0.47 | 0.21 | 0.653 | −0.077 | 17.6 |
| 3 | Soda ash | 0.47 | 0.21 | 0.786 | +0.056 | 16.5 |
| 3 | Sodium bicarbonate | 0.47 | 0.21 | 0.680 | −0.050 | 17.5 |
| 2 | Allwood Lime | 0.54 | 0.22 | 0.595 | −0.135 | 15.0 |
| 2 | Kelly Island Lime | 0.54 | 0.22 | 0.620 | −0.110 | 16.5 |
| 2 | 50–50 mixture Lehigh Lime and sodium bicarbonate | 0.65 | 0.22 | 0.625 | −0.105 | 15.5 |
| 2 | To 0.4 per cent with Lehigh Lime to 0.2 per cent with sodium bicarbonate | 0.65 | 0.20 | 0.630 | −0.100 | 14.5 |
| 4 | 50–50 mixture Lehigh Lime and soda ash | 0.52 | 0.20 | 0.973 | +0.243 | 16.4 |
| 4 | 50–50 mixture Lehigh Lime and sodium bicarbonate | 0.52 | 0.20 | 0.973 | +0.243 | 16.2 |

TABLE 5—*Continued*

| NUMBER OF CHURNINGS | NEUTRALIZER | ACIDITY OF THE CREAM AT START | ACIDITY OF THE CREAM TO CHURN | TEST OF THE BUTTERMILK | TEST OF THE BUTTERMILK ABOVE OR BELOW THE AVERAGE FOR ALL CHURNINGS | TIME TO CHURN |
|---|---|---|---|---|---|---|
| | | | | | | *minutes* |
| 2 | To 0.4 per cent with Lehigh Lime to 0.2 per cent with soda ash | 0.65 | 0.205 | 0.835 | +0.105 | 19.0 |
| 2 | To 0.4 per cent with Lehigh Lime to 0.2 per cent with sodium bicarbonate | 0.65 | 0.20 | 0.630 | −0.100 | 18.5 |
| 2 | 50-50 mixture Lehigh Lime and soda ash | 0.65 | 0.225 | 0.840 | +0.110 | 15.0 |
| 2 | To 0.4 per cent with Lehigh lime to 0.2 per cent with soda ash | 0.65 | 0.19 | 0.835 | +0.105 | 14.0 |
| 59 | Average for all | 0.539 | 0.212 | 0.730 | 0.000 | 16.4 |

below the average for all or 0.701 per cent. They varied from 0.051 to 0.121 per cent below.

The same general results are found when the groups of neutralizers are considered. The limes were below the average and the mixed and soluble groups were above. The mixed and soluble groups contained both soda ash and sodium bicarbonate.

Table 5 gives results which are comparable to those given in table 4.

The time to churn is not greatly affected by any one neutralizer.

### *Relation of the various neutralizers on the score of the fresh butter and on its keeping quality during storage*

The score of the fresh butter made with each neutralizer and the change in score during storage periods of thirty days, sixty days and ninety days are found in table 6.

In this table, the various neutralizers are grouped under lot numbers. Each churning in a lot was made from the same cream and under identical conditions except for the neutralizer.

TABLE 6

*A comparison of the scores of fresh and storage butters made with the same neutralizer*

| LOT NUMBER | NEUTRALIZER USED | SCORE OF THE BUTTER | | | | CHANGE IN SCORE FROM THE FRESH BUTTER IN | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|
| | | Fresh | Thirty days | Sixty days | Ninety days | Thirty days | Sixty days | Ninety days | |
| | Series A. Each lot made from the same cream | | | | | | | | |
| 1 | Kelly Island Lime | 88.00 | 87.50 | 86.50 | 84.00 | 0.50 | 1.50 | 4.00 | Flat, sweet flavor* |
| | Lehigh Lime | 88.00 | 87.00 | 86.00 | 83.00 | 1.00 | 2.00 | 5.00 | |
| | Allwood Lime | 88.00 | 87.50 | 86.50 | 84.00 | 0.50 | 1.50 | 4.00 | Same as in Kelly Island Lime* |
| | Peerless Lime | 88.00 | 87.00 | 86.00 | 84.00 | 1.00 | 2.00 | 4.00 | |
| | Slaked lime | 88.00 | 85.50 | 85.00 | 83.00 | 2.50 | 3.00 | 5.00 | Limy flavor |
| 2 | Kelly Island Lime | 89.50 | 89.00 | 87.50 | 85.50 | 0.50 | 2.00 | 4.00 | Sweet flavor |
| | Lehigh Lime | 89.50 | 88.50 | 87.50 | 85.00 | 1.00 | 2.00 | 4.50 | |
| | Allwood Lime | 89.50 | 88.75 | 87.00 | 85.00 | 0.75 | 2.50 | 4.50 | Flat, sweet flavor |
| | Peerless Lime | 89.50 | 88.50 | 87.00 | 84.50 | 1.00 | 2.50 | 5.00 | |
| | Slaked lime | 89.50 | 88.25 | 86.50 | 84.00 | 1.25 | 3.00 | 5.50 | |
| 3 | Lehigh Lime | 88.00 | 87.00 | 86.00 | 83.00 | 1.00 | 2.00 | 5.00 | |
| | Peerless Lime | 88.00 | 87.00 | 86.00 | 84.00 | 1.00 | 2.00 | 4.00 | |
| | Slaked lime | 88.00 | 85.50 | 85.00 | 83.00 | 2.50 | 3.00 | 5.00 | Limy flavor |
| 4 | Lehigh Lime | 89.50 | 88.50 | 87.50 | 85.00 | 1.00 | 2.00 | 4.50 | |
| | Peerless Lime | 89.50 | 88.50 | 87.00 | 84.50 | 1.00 | 2.50 | 5.00 | |
| | Slaked lime | 89.50 | 88.25 | 86.50 | 84.00 | 1.25 | 3.00 | 5.50 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | Kelly Island Lime | 88.00 | 87.50 | 86.50 | 84.00 | 0.50 | 1.50 | 4.00 | Flat, sweet flavor |
| | Allwood Lime | 88.00 | 87.50 | 86.50 | 84.00 | 0.50 | 1.50 | 4.00 | Flat, sweet flavor |
| 6 | Kelly Island Lime | 89.50 | 89.00 | 87.50 | 85.50 | 0.50 | 2.00 | 4.00 | Sweet flavor |
| | Allwood Lime | 89.50 | 88.75 | 87.00 | 85.00 | 0.75 | 2.50 | 4.50 | Flat, sweet flavor |
| Series B. Each lot made from the same cream | | | | | | | | | |
| 7 | Lehigh Lime | 89.50 | 87.00 | 86.25 | 86.00 | 2.50 | 3.25 | 3.50 | |
| | Soda ash | 88.75 | 87.50 | 86.25 | 86.00 | 1.25 | 2.50 | 2.75 | |
| | Sodium bicarbonate | 88.50 | 87.50 | 86.25 | 86.00 | 1.00 | 2.25 | 2.50 | |
| 8 | Lehigh Lime | 90.00 | 90.00 | 89.00 | 87.50 | 0.00 | 1.00 | 2.50 | |
| | Soda ash | 90.00 | 90.00 | 89.00 | 85.00 | 0.00 | 1.00 | 5.00 | Rancid in ninety days |
| | Sodium bicarbonate | 89.00 | 88.50 | 88.00 | 85.25 | 0.50 | 1.00 | 3.75 | Bicarbonate flavor |
| 9 | Lehigh Lime | 90.50 | 89.00 | 87.50 | 84.00 | 1.50 | 3.00 | 6.50 | |
| | Soda ash | 90.50 | 89.00 | 88.00 | 85.00 | 1.50 | 2.50 | 5.50 | Rancid in ninety days |
| | Sodium bicarbonate | 90.00 | 89.00 | 88.00 | 83.00 | 1.00 | 2.50 | 7.00 | Bicarbonate flavor |
| Series C. Each lot made from the same cream | | | | | | | | | |
| 10 | 50–50 mixture Lehigh Lime and soda ash | 89.50 | 88.50 | 88.00 | 86.00 | 1.00 | 1.50 | 3.50 | |
| | 50–50 mixture Lehigh Lime and sodium bicarbonate | 89.00 | 87.00 | 86.50 | 85.00 | 2.00 | 2.50 | 4.00 | Bicarbonate flavor |
| | Lehigh Lime | 90.00 | 90.00 | 89.00 | 87.50 | 0.00 | 1.00 | 2.50 | |
| | Soda ash | 90.00 | 90.00 | 89.00 | 85.00 | 0.00 | 1.00 | 5.00 | Rancid in ninety days |
| | Sodium bicarbonate | 89.00 | 88.50 | 88.00 | 85.25 | 0.50 | 1.00 | 3.75 | Bicarbonate flavor |

TABLE 6—*Continued*

| LOT NUMBER | NEUTRALIZER USED | SCORE OF THE BUTTER | | | | CHANGE IN SCORE FROM THE FRESH BUTTER IN | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|
| | | Fresh | Thirty days | Sixty days | Ninety days | Thirty days | Sixty days | Ninety days | |
| | **Series C. Each lot made from the same cream—continued** | | | | | | | | |
| 11 | 50–50 mixture Lehigh Lime and soda ash | 89.50 | 88.50 | 88.00 | 83.00 | 1.00 | 1.50 | 6.50 | Old, Rancid in ninety days |
| | 50–50 mixture Lehigh Lime and sodium bicarbonate | 89.00 | 88.00 | 87.00 | 84.00 | 1.50 | 2.00 | 5.00 | Bicarbonate flavor |
| | Lehigh Lime | 90.50 | 89.00 | 87.50 | 84.00 | 1.50 | 3.00 | 6.50 | |
| | Soda ash | 90.50 | 89.00 | 88.00 | 85.00 | 1.50 | 2.50 | 5.50 | Rancid in ninety days |
| | Sodium bicarbonate | 90.00 | 89.00 | 88.00 | 83.00 | 1.00 | 2.50 | 7.00 | |
| | **Series D. Each lot made from the same cream** | | | | | | | | |
| 12 | 50–50 mixture Lehigh Lime and soda ash | 86.50 | 84.00 | 80.00 | † | 2.50 | 6.50 | † | Rancid in sixty days |
| | To 0.4 per cent with Lehigh Lime to 0.2 per cent with soda ash | 87.50 | 85.00 | 84.00 | † | 2.50 | 3.50 | † | |
| 13 | 50–50 mixture Lehigh Lime and soda ash | 88.00 | 87.50 | 84.00 | † | 0.50 | 4.00 | † | |
| | To 0.4 per cent with Lehigh Lime to 0.2 per cent with soda ash | 89.00 | 88.00 | 85.00 | † | 1.00 | 4.00 | † | |
| 14 | 50–50 mixture Lehigh Lime and sodium bicarbonate | 87.50 | 83.00 | 77.00 | † | 4.50 | 10.5 | † | |
| | To 0.4 per cent with Lehigh Lime to 0.2 per cent with sodium bicarbonate | 89.00 | 88.00 | 85.00 | † | 1.00 | 4.00 | † | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 15 | 50–50 mixture Lehigh Lime and sodium bicarbonate | 87.00 | 84.00 | 81.00 | † | 3.00 | 6.00 | † | |
| | To 0.4 per cent with Lehigh Lime to 0.2 per cent with sodium bicarbonate | 87.25 | 86.00 | 85.00 | † | 1.25 | 2.25 | † | |
| Series E. Each lot made from the same cream | | | | | | | | | |
| 16 | 50–50 mixture Lehigh Lime and soda ash | 89.50 | 88.50 | 87.00 | 85.00 | 1.00 | 2.50 | 4.50 | |
| | 50–50 mixture Lehigh Lime and sodium bicarbonate | 88.50 | 87.00 | 86.75 | 86.00 | 1.50 | 1.75 | 2.50 | Bicarbonate flavor |
| 17 | 50–50 mixture Lehigh Lime and soda ash | 89.50 | 88.50 | 88.00 | 86.00 | 1.00 | 1.50 | 3.50 | |
| | 50–50 mixture Lehigh Lime and sodium bicarbonate | 89.00 | 87.00 | 86.50 | 86.00 | 2.00 | 2.50 | 3.00 | Bicarbonate flavor |
| 18 | 50–50 mixture Lehigh Lime and soda ash | 89.50 | 88.50 | 88.00 | 83.00 | 1.00 | 1.50 | 6.50 | Rancid after ninety days |
| | 50–50 mixture Lehigh Lime and sodium bicarbonate | 89.00 | 88.00 | 87.00 | 84.00 | 1.00 | 2.00 | 5.00 | |
| 19 | To 0.4 per cent with Lehigh Lime to 0.2 per cent with soda ash | 88.50 | 85.00 | 84.00 | † | 3.50 | 4.50 | † | |
| | To 0.4 per cent with Lehigh Lime to 0.2 per cent with sodium bicarbonate | 88.25 | 86.00 | 85.00 | † | 1.25 | 3.25 | † | |
| 20 | To 0.4 per cent with Lehigh Lime to 0.2 per cent with soda ash | 89.00 | 88.00 | 85.00 | † | 1.00 | 4.00 | † | |
| | To 0.4 per cent with Lehigh Lime to 0.2 per cent with sodium bicarbonate | 89.00 | 88.00 | 85.00 | † | 1.00 | 4.00 | † | |

* Mottled after ninety days.

† Discontinued after sixty days storage because of poor quality.

The table is divided into five series. Each one contains the results secured with certain groups of neutralizers in the same cream. They are arranged so that comparisons are interchangeable between series, for a certain neutralizer is found in more than one.

Since one cream may produce a better quality butter than another, even though they both may appear to be similar as determined by taste and smell, the cream used in this series of determinations was selected so as to minimize this condition. The cream was taken from that received at a large commercial creamery, and was of fairly good quality. The acidity varied from 0.47 to 0.65 per cent and the butterfat from 29 to 34 per cent. The acidity was reduced to approximately 0.2 per cent and no starter was added.

The results show that there was very little advantage gained as far as the quality of the fresh butter was concerned by the use of one neutralizer over the other when each was used by itself. But when a combination was used, either together or separately, the score of the fresh butter was not as high as when a single neutralizer was used. On the other hand, when the acidity was reduced to 0.4 per cent with lime and then to 0.2 per cent with soda ash or sodium bicarbonate, the quality of the fresh butter was better than when the two were mixed before they were added to the cream.

In comparing the use of lime and soda ash or sodium bicarbonate for reducing the acidity of the cream, the same general quality of cream was used as with all other neutralizers. Many creameries make a practice of using one certain neutralizer except for cream which is extremely high in acid, as 0.8 per cent and above. In such cases, the acidity is reduced to a given point with lime and the balance with a soda ash or a sodium bicarbonate solution. This method does tend to eliminate a limy or soapy flavor which would ordinarily occur, if one neutralizer was used.

The limes and soda ash produced fresh butter which was similar in quality. The sodium bicarbonate butter scored slightly lower than the soda ash or the limes because of a bicarbonate flavor which was detected in the butter whenever it was used. The mixtures of lime and the soluble neutralizers resulted in a lower score than when only one was used.

Another interesting fact is brought out in table 6. In each series where soda ash and sodium bicarbonate were used in the same cream, either separately or with lime, the fresh butter made with the former scored higher than that made with the latter. It seems impossible to make butter from cream neutralized with sodium bicarbonate without securing the bicarbonate flavor in the fresh butter. After storage periods of thirty, sixty and ninety days, the butter was scored again by the same judge. The reduction in score for the soda ash was greater than for the sodium bicarbonate in each series. In most cases the score of the fresh butter made with sodium bicarbonate was a quarter to one point lower than that made with soda ash, but after sixty to ninety days, the score was the same or above that made with soda ash. This would tend to indicate that butter made with soda ash does not retain its quality during storage as well as that made with sodium bicarbonate. In each case where rancidity developed during storage, soda ash had been used as the neutralizer. Possibly, this can be explained in that soda ash is a stronger alkali and causes a more rapid chemical breakdown of the butterfat. More data are necessary before positive conclusions can be drawn stating that butter made with soda ash as a neutralizer, will not store as well as that made with sodium bicarbonate.

The neutralizers did not have any effect on the body of the butter except in one series. In lot 1, mottles were noticed where Kelly Island Lime and Allwood Lime had been used. The cause was not determined, although this condition was not noticeable until after ninety days storage.

The butter made with a combination (lots 12, 13, 14, 15, 19 and 20) of neutralizers was not scored after sixty days as they were discarded because the scores had dropped to 85 or below. The others were held for ninety days.

In comparing the various limes, it can not be said that one has an advantage over the other as far as score is concerned. Series

A shows that the fresh butter for each lot was the same. In thirty and sixty days time all were practically the same except the slaked lime, and it was slightly lower and showed a limy flavor. Another outstanding fact was that a flat, sweet flavor was noticed in each case where a magnesium lime had been used. Although this flavor was present, it did not lower materially the score as compared with the calcium limes. From the data obtained, it can be said that no one lime has an advantage over another if each is handled properly.

In series B experiments, the soluble neutralizers were compared with lime. The data show that neither has a decided advantage over the other. As far as the fresh butter was concerned, all were the same except for the flavor found when sodium bicarbonate was used, which reduces the score slightly. After storage (three lots), no one neutralizer seemed to be best. In one case, the lime held up best; in another, the soda ash; and in the third, the sodium bicarbonate.

## SUMMARY

1. A better quality of fresh butter and a more exhaustive churning was secured with a 5 per cent solution of soda ash or sodium bicarbonate, and a 10 per cent solution of a lime than with a stronger solution of these neutralizers.
2. The time required for a complete reaction between the acid in the cream and the neutralizer after its addition, varied with the neutralizers. The soluble neutralizers reacted completely in two minutes; the calcium limes in five minutes; and the magnesium limes in ten to fifteen minutes.
3. The reduction in acidity during pasteurization varied with the neutralizer. The maximum reduction with the soluble neutralizers was 0.75 per cent; with the calcium limes, 0.04 per cent; and with the magnesium limes, 0.058 per cent. The reduction seemed to depend on the solubility of the neutralizer and also upon the formation of carbon dioxide gas which was driven off by heat.
4. No definite rule can be formulated as to the effect the neutralizers had on the time of churning.

5. The use of soda ash increased the fat lost in the buttermilk; while the sodium bicarbonate and the limes gave a more exhaustive churning with the latter more efficient than the former.

6. No one of the neutralizers tried (lime, soda ash or sodium bicarbonate) can be said to produce the best quality of fresh butter. The sodium bicarbonate tended to reduce the score slightly because of a slight bicarbonate flavor which was always present in the butter.

7. The separate neutralizers gave a higher score butter than when two were used together, either separately or mixed before they were added to the cream.

8. When lime and soda ash or sodium bicarbonate were used, the best results were gained by using them separately rather than mixing them, with the lime used first.

9. When soda ash entered into the neutralizer, either by itself or with lime, the butter was of a higher quality at the start but it did not hold up as well in storage as did the butter in which sodium bicarbonate was used as a neutralizer, either by itself or with lime.

10. In each case where rancidity developed after storage, soda ash had been used as the neutralizer.

11. No one lime had an advantage over the others, either for fresh or storage butter, when all were handled properly.

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# RELATIVE RATES OF SECRETION OF VARIOUS MILK CONSTITUENTS*

W. L. GAINES

*Department of Dairy Husbandry, University of Illinois, Urbana, Illinois*

If we take a unit quantity of natural cow's milk, that is, the entire milk as obtained from the cow during a period of some length under normal conditions, we know that it may be separated on a chemical basis into several component parts. The constituents of milk customarily determined by the chemist, are, in order of their average magnitude per unit of milk: water, lactose, fat, protein and ash. A great many analyses of milk have been made on this basis, from various points of interest. The chemist has been interested from the standpoint of the composition of the milk; the food inspector, from the standpoint of food values, abnormalities or adulterations of the milk; and the biologist, from the standpoint of the physiological mechanism and requirements of milk secretion. From the latter standpoint the work of T. L. Haecker in formulating a feeding standard for dairy cows constitutes a notable example.

Haecker (1) has published the analyses of 543 samples of milk and derived certain conclusions from the data particularly with reference to the feed requirements for milk production. The analyses give the per cent of fat, protein, lactose, ash and dry matter. Fat was determined in part gravimetrically and in part by the Babcock method; protein, dry matter and ash gravimetrically; lactose, by difference. Each of the 543 samples represents the milk of an individual cow for one week, fourteen milkings. The samples were taken at various stages of lactation and under various conditions of experimental feeding, reference especially to the nutritive ratio of the ration. Fifty-two cows of various ages, weights and breeding are represented: ages, two to fourteen years; weights, 600 to 1350 pounds; breeding, natives and pure-

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* Received for publication August 20, 1925.

breds, grades or cross-breds of the Ayrshire, Brown Swiss, Guernsey, Hereford, Holstein, Jersey and Shorthorn breeds. Altogether, these analyses constitute a unique and valuable body of accurate data on the constituents of cows' milk and it has seemed desirable to study the relations which they show by the usual statistical methods.

## CORRELATION BETWEEN THE MILK CONSTITUENTS

In correlating the percentage values of the several constituents class intervals were used as follows: water, 0.5; fat, 0.2; protein, 0.2; lactose, 0.1; ash, 0.02. The means, standard derivations and coefficients of variability are given in table 1. The significance of these constants is practically limited to the present data because of the throwing together of several breeds in very dissimilar porportions of the total, as to numbers. The coefficients of variability in fat, protein, lactose and ash are about double what we should expect to find in any one breed (cf. Yapp (2)). As measured by this coefficient water is by far the least variable of the five constituents of the milk as determined, while fat is the most variable. The standard deviation of the water, however, exceeds that of the fat.

TABLE 1

*Values of the percentage content of the various constituents of the 543 weekly milk yields*

| CONSTITUENT | MEAN | STANDARD DEVIATION | COEFFICIENT OF VARIABILITY |
|---|---|---|---|
| Water........................ | 86.303±0.044 | 1.524±0.031 | 1.77±0.04 |
| Lactose...................... | 4.888±0.007 | 0.259±0.005 | 6.65±0.11 |
| Fat.......................... | 4.731±0.029 | 0.998±0.020 | 21.10±0.43 |
| Protein...................... | 3.349±0.014 | 0.488±0.010 | 14.57±0.30 |
| Ash.......................... | 0.723±0.001 | 0.049±0.001 | 6.84±0.14 |

It is possible to compute ten simple correlations between the five constituents determined. These are given in table 2 in order of the absolute magnitude of the coefficients. It may be noted that there is a great difference in the values of the coefficients, three of them being remarkably high, while all the others are rather low, although statistically significant as judged by the probable errors. It may be noted also that the four coefficients involving water are all negative while those between the solid constituents are all positive except that between lactose and ash.

TABLE 2

*Correlation between the relative average rates of secretion of the various milk constituents for the 543 weekly periods*

| VALUES CORRELATED | COEFFICIENTS OF CORRELATION |
|---|---|
| Fat per cent and water per cent | $-0.966 \pm 0.002$ |
| Protein per cent and water per cent | $-0.885 \pm 0.006$ |
| Protein per cent and fat per cent | $+0.812 \pm 0.010$ |
| Lactose per cent and water per cent | $-0.386 \pm 0.025$ |
| Protein per cent and ash per cent | $+0.325 + 0.026$ |
| Ash per cent and water per cent | $-0.264 \pm 0.027$ |
| Fat per cent and lactose per cent | $+0.263 \pm 0.027$ |
| Fat per cent and ash per cent | $+0.232 \pm 0.027$ |
| Protein per cent and lactose per cent | $+0.149 \pm 0.028$ |
| Lactose per cent and ash per cent | $-0.140 \pm 0.028$ |

TABLE 3

*Interrelational equations for several milk constituents, derived from the constants of Tables 1 and 2*

$a$ = ash per cent; $f$ = fat per cent; $l$ = lactose per cent $p$ = protein per cent; $s$ = solids per cent; $s\text{-}n\text{-}f$ = solids-not-fat per cent; $w$ = water per cent.

The probable errors are for a single variate.

| BASED ON FAT PER CENT | BASED ON PROTEIN PER CENT |
|---|---|
| $a = (0.671 + 0.011\mathrm{f}) \pm 0.032$ | $a = (0.613 + 0.033\mathrm{p}) \pm 0.032$ |
| $p = (1.46 + 0.40\mathrm{f}) \pm 0.19$ | $f = (1.66\mathrm{p} - 0.83) \pm 0.39$ |
| $l = (4.56 + 0.07\mathrm{f}) \pm 0.17$ | $l = (4.62 + 0.08\mathrm{p}) \pm 0.17$ |
| $s = 6.74 + 1.47\mathrm{f}$ | $s = 4.45 + 2.76\mathrm{p}$ |
| $s\text{-}n\text{-}f = 6.74 + 0.47\mathrm{f}$ | $w = (95.55 - 2.76\mathrm{p}) \pm 0.48$ |
| $w = (93.26 - 1.47\mathrm{f}) \pm 0.27$ | |

## THE REGRESSION EQUATIONS

The regression equations, computed from the constants of tables 1 and 2 are given in table 3 with reference to the regression of the several constituents on fat percentage and protein percentage. Figures 1 and 2 show graphically the curves of the equations and the mean observed values. It is apparent from the plotted values that the regressions are substantially linear in

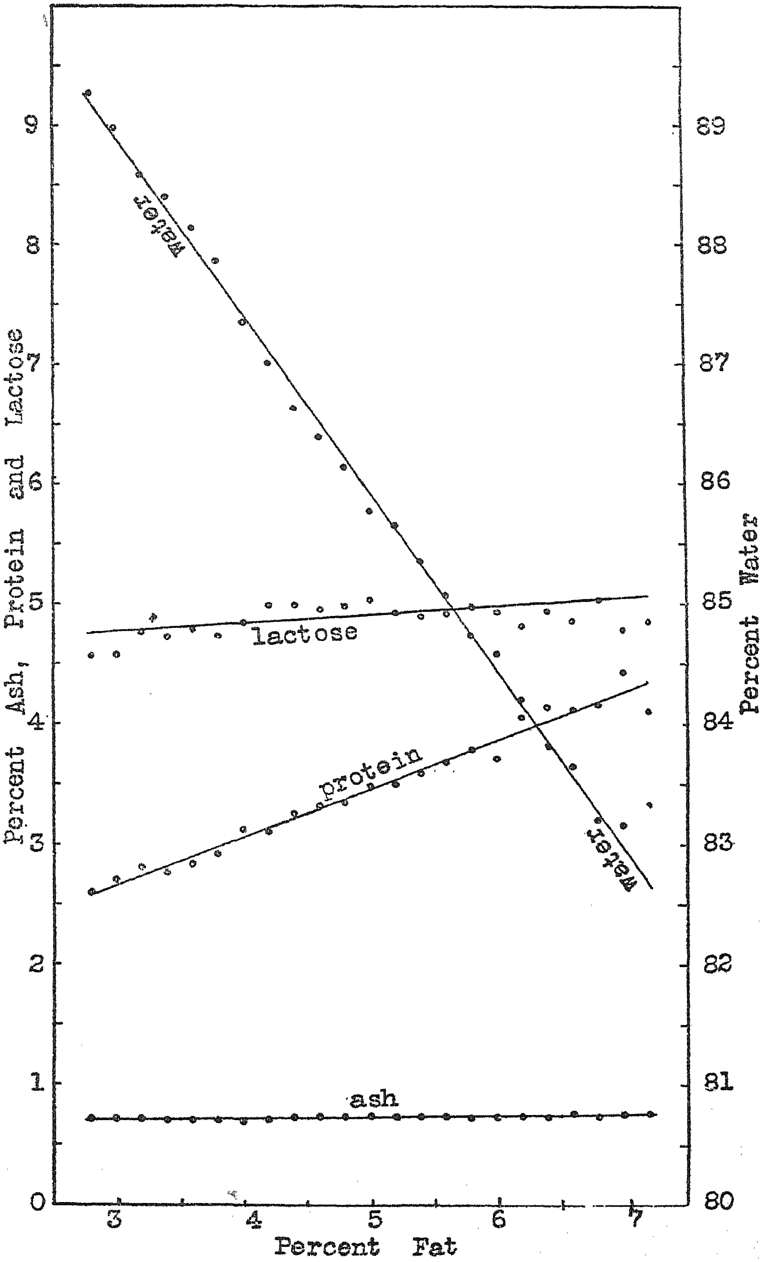

FIG. 1. REGRESSION OF ASH, PROTEIN, LACTOSE AND WATER ON FAT. EQUATIONS TO STRAIGHT CURVES IN TABLE 3

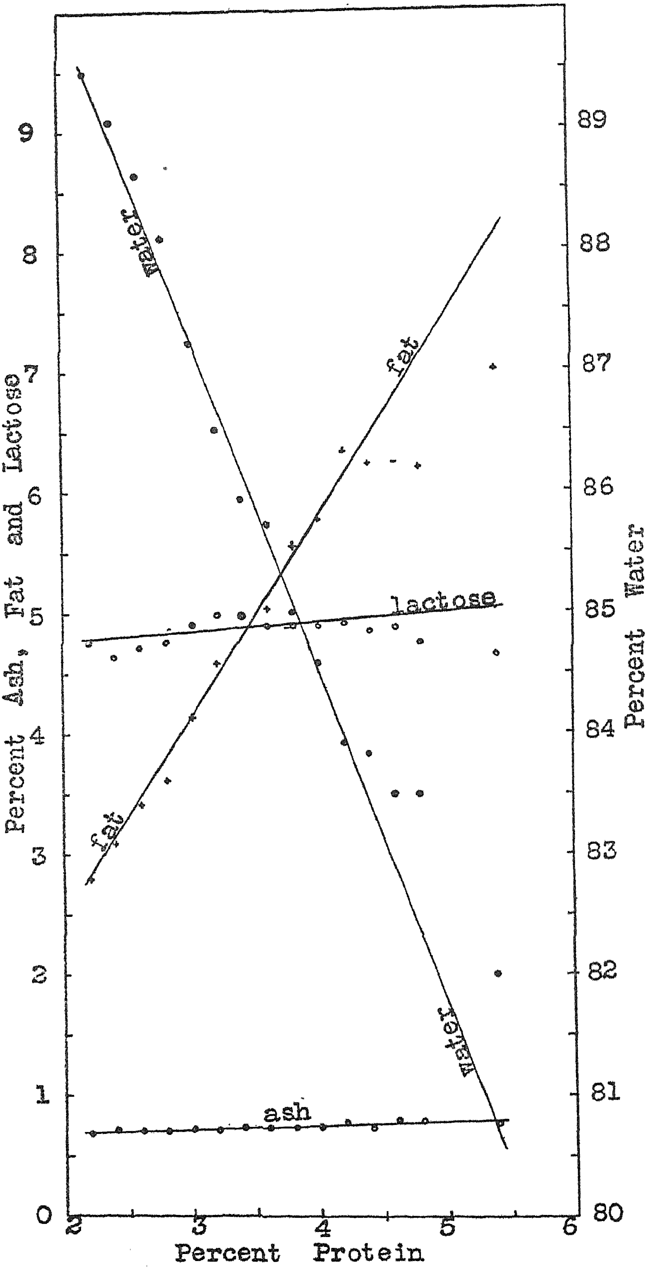

FIG. 2. REGRESSION OF ASH, FAT, LACTOSE AND WATER ON PROTEIN. EQUATIONS TO STRAIGHT CURVES IN TABLE 3

all cases, although that for lactose seems to have a slight tendency to a concave downward curvature. It seems also that in general there is a tendency for the regressions on fat percentage to be rather more regular than on protein percentage.

The equation, $p = (1.46 + 0.40f) \pm 0.19$ is of particular interest. Timpe (3) from analyses of milk from 21 cows of various breeds derived the relation $p = 2 + 0.35f$. Andersen and Langmack (4) from 1080 analyses of the milk of Red Danish cows derived the equation $p = 1.597 + 0.446f$. They have rounded this into $p = 1.4 + 0.5f$, thus increasing the slope or ratio of protein change to fat change. From the evidence available it would seem that their ratio was already somewhat high as expressing the general relation (cf. fig. 3) and might better have been decreased rather than increased.

## RELATIVE RATES OF SECRETION OF VARIOUS CONSTITUENTS

The records of composition as previously stated, represent for any one sample the entire milk produced by an individual cow during seven consecutive days. While they give no information as to the *absolute* amount of milk or its several constituents secreted during the period, they do show the *relative* amounts of the several constituents of the milk secreted during the period. If any two constituents of milk are intimately related in the mechanism of milk secretion, so that when the rate of secretion of one increases the rate of the other increases accordingly then values of these two as expressed in percentage composition of the various samples of milk will tend to bear to each other a constant ratio. Under these conditions the data as above treated would give a high positive correlation between any two constituents so related. Conversely, a high positive correlation between any two constituents indicates that the two are in some manner intimately connected in the physiological processes of secretion.

A high negative correlation need not have a similar meaning. If we consider the solids and water of milk obviously $r_{sw}$ must equal $-1$. If the secretion of each of the five milk constituents we are considering proceeds entirely independently then we should expect to find a negative correlation between any two constit-

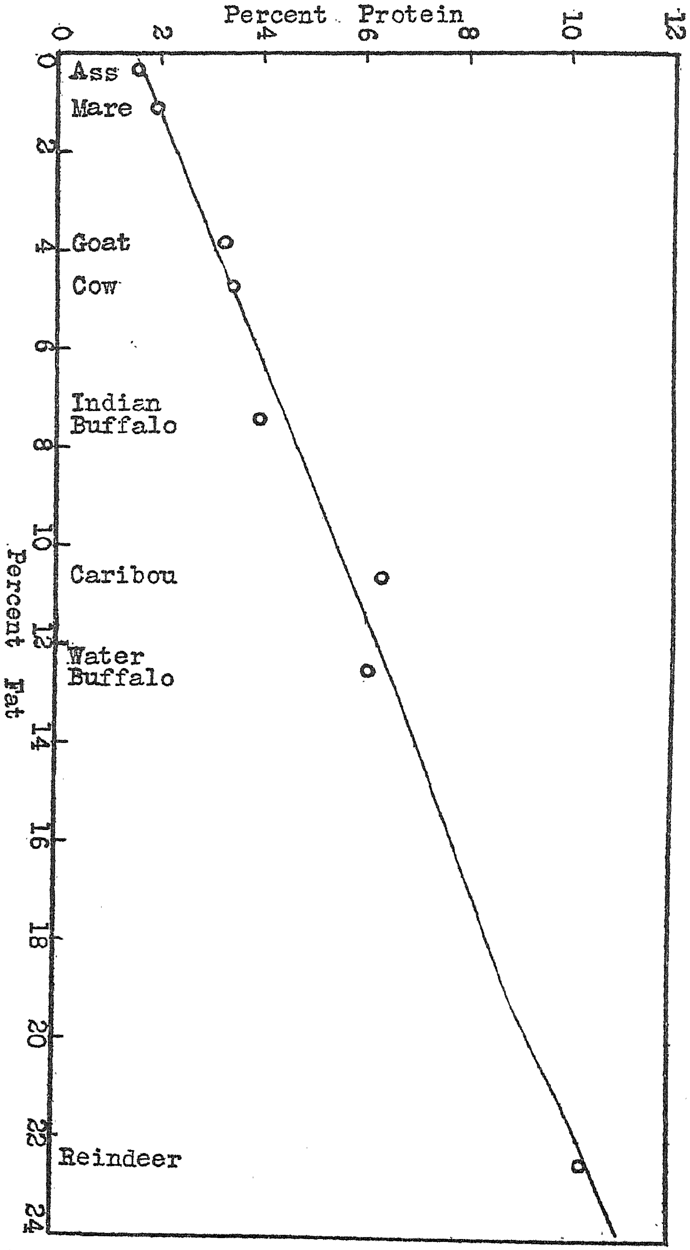

FIG. 3. RELATION OF PROTEIN TO FAT IN THE MIXED MILK OF EIGHT SPECIES OF MAMMALS

The straight curve expresses the relation as found between individual cows uents. Consequently, the negative correlations do not have the same significance as the positive correlations.

It will be understood, of course, that the chemical distinctions made in the series of analyses under discussion are not necessarily sufficiently fine to show all of the physiological relations that may be susceptible of elucidation through quantitative chemical analysis. One of the five constituents represents a single compound, that is, water. Lactose perhaps represents also a distinct compound but as determined, by difference, there is some uncertainity as to this. The other three represent more or less complex mixtures.

The most significant positive correlation is that between fat and protein. The secretion of the materials involved in these two catagories must be quite intimately related. Further, this relation seems to be a general phenomenon of milk secretion since substantially the same relation between the rate of fat secretion and protein secretion is found between widely separated species of mammals. It is true that a great many analyses of milk of various mammals as reported in the literature do not show the same relation as shown above for the cow. For the comparison under study it is necessary, however, to be somewhat discriminating about how well the sample analysed actually represents the total amount of milk secreted by a particular individual or group of individuals during a certain period. Milk is very subject to disturbance in homogeneity with respect to its fat content. The

TABLE 4

*Relation of fat and protein in the milk of several species of mammals—average data selected as representative of the normal secretion*

| SPECIES | PER CENT FAT | PER CENT PROTEIN | AUTHORITY |
|---|---|---|---|
| Ass | 0.36 | 1.57 | Schlossman (5) |
| Mare | 1.09 | 1.89 | Vieth (6) |
| Goat | 3.82 | 3.21 | Bosworth and Van Slyke (7) |
| Cow | 4.73 | 3.35 | Haecker (1) |
| Indian buffalo | 7.41 | 3.89 | Leather (8) |
| Caribou | 10.63 | 6.31 | Musgrave and Richmond (9) |
| Water buffalo | 12.46 | 6.04 | Levine (10) |
| Reindeer | 22.46 | 10.30 | Barthel and Bergman (11) |

force of gravity or passage of the milk through the fine spaces of the gland readily alter the fat concentration in the various fractions of the milk. For this reason many fat determinations are undoubtedly very misrepresentative of the true activity of the mammary gland. If the fat determination is not representative the fat-protein ratio is, of course, misleading. Among those mammals which are regularly milked as a commercial source of milk we may expect to be able to obtain samples which are truly representative of the activity of the mammary gland. While there are no data for mammals other than the cow comparable with that of Haecker in extent and reliability, the analyses summarized in table 4 seem from the authors' accounts to be fairly representative of the normal secretion.

The data of table 4 are given graphically in figure 3 together with the curve, derived from Haecker's analyses of milk from individual cows. It is certainly very striking that this relation between fat and protein should hold so closely in other species and through so wide a range of fat percentage—0.36 per cent in the ass to 22.46 per cent in the reindeer. It gives greater significance to the relation in connection with the general physiology of milk secretion.

The high negative correlation between water and fat is to be interpreted as indicating that the secretion of water and fat are not physiologically connected. The same interpretation is to be applied to the high negative correlation between water and protein. That is, the secretion of water is largely independent of the secretion of fat or protein, where the general level of activity of the gland is constant.

The same line of reasoning requires the interpretation that the secretion of water and the secretion of lactose and ash are interdependent—otherwise water should show a negative correlation of substantially the same magnitude in the case of each of the four other constituents.

A more refined and detailed chemical analysis is required to give an acceptable picture of the relations between water, lactose, and ash secretion. For, it is apparent from the work of Jackson and Rothera (12) that a sufficiently fine distinction in the determination of the ash constituents would show a high negative correlation between lactose and portions of the ash. The gross distinctions of the present analyses can be no more than suggestive of fundamental relationships.

## SUMMARY

Chemical analyses of 543 samples of milk of individual cows of various breeds published in Minnesota Bulletin 140 are analysed statistically. The results are interpreted physiologically as showing the relative ratios of secretion of the several constituents at a constant level of activity of the mammary gland. The secretion of fat and protein appear to be intimately related; $r_{fp} = 0.812 \pm 0.010$. The secretion of water and the secretion of fat or protein are largely independent on the basis that entire independence would result in a high negative coefficient of correlation;

$$r_{wf} = -0.966 \pm 0.002 \text{ and } r_{wp} = -0.885 \pm 0.006$$

The equation for protein per cent ($p$) based on fat per cent ($f$) is $p = (1.46 + 0.40f) \pm 0.19$. This relation appears to hold very closely also between several species, covering a range of fat percentage from 0.36 to 22.46.

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# KEEPING QUALITY OF SWEET CREAM BUTTER AS AFFECTED BY TEMPERATURE OF PASTEURIZATION OF THE CREAM

WILLIAM WHITE AND GEORGE R. CAMPBELL[1]

*Bureau of Dairying, United States Department of Agriculture, Washington, D. C.*

Butter made from unripened pasteurized sweet cream, commonly termed sweet cream butter, has won the approval of manufacturers, dealers, and consumers because of its fine, mild flavor, its uniformity, and its superior keeping quality. Butter of this type is made largely in creameries where the vat method of pasteurization is used. The prevailing temperature is 145°F. and the time of heating thirty minutes, though in some plants higher temperatures have been used.

In order to determine the effect of the pasteurizing temperature on the keeping quality of sweet cream butter, cream was pasteurized at the following temperatures and samples of the butter were held in storage at about 0°F. for seven and one-half to nine months:

145°F. for 25 minutes
145°F. for 30 minutes
150°F. for 30 minutes
155°F. for 30 minutes
145° to 165°F. for 30 minutes

In pasteurizing at 145° to 165°F. for thirty minutes the cream was heated in the usual way to 145°F., then heated more slowly so that thirty minutes was required to raise the temperature to 165°F. It was then cooled as quickly as possible to churning temperature.

One hundred and sixteen churnings were made between May 23 and August 19, 1924, at two creameries in Minnesota that were making butter on a Navy contract. The different pasteurizing temperatures were used on different vats of cream; the cream was closely graded and the vats of cream were so uniform that using split batches was not considered necessary.

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[1] The authors wish to express their appreciation of the assistance in this work of A. Kristensen and Frank W. Logan, Navy butter supervisors; O. O. Vaughn, operator of the Brownton Coöperative Creamery, Brownton, Minnesota, and W. B. Steinhouse, operator of the New Richland Coöperative Creamery, New Richland, Minnesota.

All the samples used were from churnings accepted on the Navy contract and all were scored when fresh 95 points on a basis of sweet cream butter.

All samples were packed in 5-pound lacquered tin cans and were shipped with other butter in refrigerator freight cars to the Naval Operating Base, Hampton Roads, Virginia, where they were immediately placed in cold storage and kept there until April 1, 1925.

TABLE 1

| PASTEURIZING TEMPERATURE |  | NUMBER OF CHURNINGS | ACIDITY OF CREAM, AVERAGE | WATER IN BUTTER, AVERAGE | SALT IN BUTTER, AVERAGE | AVERAGE SCORE AFTER STORAGE |
|---|---|---|---|---|---|---|
| Degrees Fahrenheit | Minutes |  |  |  |  |  |
| Creamery no. 1 |  |  |  |  |  |  |
|  |  |  | *per cent* | *per cent* | *per cent* |  |
| 145 | 25 | 8 | 0.1810 | 13.4 | 2.93 | 94 |
| 145 | 30 | 8 | 0.1808 | 13.4 | 2.92 | 94 |
| 150 | 30 | 9 | 0.1850 | 13.41 | 3.01 | 94 |
| 155 | 30 | 11 | 0.1902 | 13.33 | 2.96 | 94 |
| 145–165 | 30 | 6 | 0.1880 | 13.47 | 2.96 | 94 |
| Creamery no. 2 |  |  |  |  |  |  |
| 145 | 25 | 17 | 0.191 | 13.38 | 2.93 | 92.94 |
| 145 | 30 | 12 | 0.202 | 13.39 | 2.97 | 92.83 |
| 150 | 30 | 14 | 0.195 | 13.40 | 2.93 | 93.00 |
| 155 | 30 | 15 | 0.201 | 13.36 | 3.02 | 93.00 |
| 145–165 | 30 | 16 | 0.181 | 13.39 | 3.03 | 93.06 |

After they were out of storage for one day they were scored by a commercial judge of recognized ability.

Table 1 shows the scores of the samples for the different pasteurizing temperatures, also the acidity of the cream and the water and salt content of the butter.

This table shows that the samples of butter from Creamery no. 1 after storage were so uniform in quality that they all received the same score; at the other creamery the difference in score was so slight that it does not indicate the superiority of one group of samples over another.

## SUMMARY

In the manufacture of 116 churnings of sweet cream butter for storage, cream was pasteurized at 145°F. for twenty-five minutes, 145°F. for thirty minutes, 155°F. for thirty minutes, and 145° to 165°F. for thirty minutes. Samples of the butter were stored at 0°F. for seven and one-half to nine months. Scores of the butter after storage indicate the keeping quality was not influenced by these temperatures of pasteurization of the cream.

# RELATION BETWEEN THE GOLD NUMBER OF GELATIN AND ITS VALUE IN THE ICE CREAM MIX*

H. C. MOORE, W. B. COMBS, AND C. D. DAHLE

*Division of Dairy Husbandry, University of Minnesota, St. Paul, Minnesota*

The gold number has been used by chemists for twenty-five years as a means of measuring the protective property of colloids, but this method has never been applied to the grading of gelatin to be used in the manufacture of ice cream. Thomas (6) (1925) says, "The gold numbers are useful, however, solely as very rough indices of the relative protective powers, because the gold number of a given protective colloid depends so largely upon many conditions." Elliott and Sheppard (3) (1921) studied the gold numbers of seventeen different samples of commercial gelatins and concluded from their study that the classification made possible by the gold numbers was too rough, and moreover, did not bear any simple relation to those properties of chief interest to users of gelatins. Their experiments were mainly on the factors that affect the gold number determinations and they did not study the properties and characteristics of the gelatin.

*Plan of experiment.* It is recognized by the authors that the gold number is too technical a test to be carried out by the practical ice cream maker. It was thought, however, that a relation might exist between the gold numbers of the commercial gelatins and one or more of the other tests that are now being used by the ice cream manufacturer. The gold number of the gelatins studied was compared with the hydrogen-ion concentration, ash and moisture content, bacterial count, swelling

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* Received for publication July 8, 1925.

Published with the approval of the Director as paper No. 549, Journal Series, Minnesota Agricultural Experiment Station. The data are taken from a thesis presented by H. C. Moore in partial fulfillment of the requirements for the degree of M. S., University of Minnesota.

strength, solubility, gel strength and jelly value. A study was made of the effect of each gelatin on the viscosity of the ice cream mix, retention of air by the frozen mixes, standing up qualities or melting resistance of the finished ice cream, and the effect of the gelatins on the flavor and texture of the ice creams after hardening.

*Selection of gelatins.* Six samples of gelatin were selected for this investigation. In selecting the gelatins an effort was made to obtain one very high grade and one poor grade gelatin. The gelatins were selected on the basis of grade and quality, without any consideration of the brand. In this investigation the gelatins will be referred to by the numbers 1 to 6 inclusive.

*Experimental methods.* The gold numbers were determined by using the Zsigmondy's (7) red gold sol, prepared by the formaldehyde method. Two methods were used in determining the gold numbers. The one recommended by Elliott and Sheppard (3) (1921) and the other recommended by the Division of Biochemistry of the University of Minnesota. These two methods checked within the range of experimental error. Therefore, only the average results are reported in this paper.

The pH of the gelatins was determined on a Leeds-Northrup Type K potentiometer using a 0.78 per cent gelatin solution. The 0.78 per cent gelatin solution represents the concentration of gelatin in the water phase of an ice cream mix containing 36.5 per cent total solids. The moisture content was determined with the Mojonnier Milk Tester. The ash content was determined by heating 1 to 2 grams of the gelatin in platinum crucibles, at a red heat. The bacterial counts were made by plating a 1:100 solution of the gelatin on plain agar. The swelling strength was determined by adding 5 grams of gelatin to a 100 cc. cylinder and adding enough water at 21°C. to make 100 cc. The gelatin was stirred for one minute to prevent lumping. After the gelatin had soaked for ten minutes the cubic centimeter of swelling was observed. The gelatin was then dissolved by placing the cylinders in a water bath at 60°C. The gelatin was stirred until completely dissolved, and the time in seconds that it took the gelatin to dissolve was used as the rate of solubility.

The gel strength was determined by the Burke Gelatin Standardizer following the method recommended by Burke (1) the originator (1923). The jelly value is represented by the smallest amount that will gel in an aqueous solution. The samples were dissolved and held at 10°C. for thirty minutes before the reading was taken.

The ice cream mixes used in this study were calculated to contain 12 per cent fat, 10 per cent serum solids, 14 per cent sugar, and from 0.3 to 0.5 per cent gelatin. All of these ingredients, with the exception of the gelatin, were mixed together and pasteurized at 63°C. for thirty minutes. The mix was cooled to 110°F. and homogenized at 2000 pounds pressure. The mix was then divided into seven lots. To six of these lots was added one of the gelatins used in this investigation. The seventh lot served as the check mix. Each lot was cooled to 20°C. at which time the samples were taken for viscosity studies, after which the mix was aged 24 hours in the cold room at about 20°C.

The viscosity tests were determined by means of a MacMichael viscosimeter. Standardized wires, standardized in the laboratories of the Dairy Division, were used. The 3 cm. disc bob of the MacMichael was used, and the results reported in MacMichael degrees converted in terms of No. 30 wire.

The ice cream mixes were frozen in a small batch freezer in which it was possible to freeze five different mixes under identical conditions. The time of freezing process varied from twelve to fifteen minutes for different experiments. The temperature of the brine used varied from 11°C. to 12°C.

The retention of air by the ice cream mixes was measured by filling a 100 cc. cylinder with the mix from the freezer. These cylinders were placed in a cold room at 2°C. over night and the next morning they were placed at room temperature and the time noted when the air left the different samples.

To measure the standing up qualities and the melting resistance of the ice creams, pint paper cartons were filled with the mix when frozen and placed in the hardening room at about −18°C. These samples were allowed to harden over night and the next morning they were placed on a wire gauze over a tall one liter beaker at room temperature (22°C.). Photographs of the ice creams were taken at the end of two and three hours.

*Experiment I.* Comparison of gold number to H-ion concentration and various common tests used in selecting gelatin for ice cream manufacture. In recent years the ice cream manufacturer has selected gelatin by means of moisture and ash content, bacterial count, solubility, gel strength, and jelly value. The present day manufacturer is giving more attention to gel strength and bacterial count. The object of this experiment was to make a comparison of the gold number of the H-ion concentration and various common tests used in selecting gelatin. The data are presented in table 1.

TABLE 1

*Relation of gold number to H-ion concentration and to common tests used in selecting gelatin by the ice cream manufacturer*

| SAMPLE NUMBER | GOLD NUMBER | pH OF 0.78 PER CENT GELATIN SOLUTION | ASH | MOISTURE | BACTERIA | SWELLING | SOLUBILITY | GEL STRENGTH BURKE METHOD | JELLY VALUE |
|---|---|---|---|---|---|---|---|---|---|
| | | | *per cent* | *per cent* | *per gram* | *cc.* | *seconds* | | *per cent* |
| 1 | 0.0048 | 5.647 | 1.829 | 13.27 | 5,600 | 33 | 180 | 82.0 | 1.4 |
| 2 | 0.0040 | 6.002 | 0.793 | 10.99 | 13,500 | 39 | 150 | 82.7 | 1.8 |
| 3 | 0.017 | 4.334 | 0.790 | 11.08 | 600 | 48 | 165 | 81.0 | 2.0 |
| 4 | 0.020 | 4.809 | 1.278 | 11.46 | 7,300 | 51 | 145 | 74.0 | 2.2 |
| 5 | 0.013 | 5.618 | 2.819 | 13.85 | 3,500 | 47 | 125 | 74.3 | 2.2 |
| 6 | 0.023 | 3.861 | 0.964 | 11.33 | 300 | 80 | 210 | 84.3 | 1.8 |

The smallest gold number represents the highest protective power. Gelatin has been found by all investigators to have the greatest range in gold numbers of any colloid. The range given by Zsigmondy (7) (1917) was from 0.01 to 0.005 and by Gortner (4) (1920) 0.0125 to 0.005. The range found in this experiment was from 0.023 to 0.004. The gold number 0.004 is lower than has been reported by any investigator with gelatin. There was found to be no direct relationship between the gold numbers and any of the other tests used in studying these gelatins.

The gelatin with the lowest gold number had the highest bacterial content and the gelatin with the lowest bacterial content had the poorest gold number, but the other samples did not follow in order. The ash content seemed to have no direct effect upon the gold number. The swelling strength, with the exception of samples 1 and 2, was very close to an inverse relationship with the gold numbers. The gelatin with the highest strength by the Burke Gelatin Standardizer had the poorest gold number and the sample second highest in strength had the lowest gold number. The gelatin with the highest viscosity had the second best gold number, and the gelatin with the second best viscosity had the poorest gold number. Two of the gelatins had the same jelly value and these two gelatins also had the highest and lowest gold number. With one exception, there was a direct relationship between the gold numbers of these gelatins and the H-ion concentration of the gelatins. Investigators agree that the H-ion concentration affects the gold number determination and this investigation bears out this conclusion.

Thomas (5) (1924) found that if the protective colloid solution is slightly acid it will show a poorer protective action than one which is neutral or slightly alkaline. In this investigation the gelatin with the highest pH had the lowest gold number, and the gelatin with the poorest gold number was found to be the most acid gelatin. Samples 2 and 4 were very close to the isolectric point of gelatin and this may account for the fact that while sample 4 is the more alkaline of the two, yet it has the poorer gold number. While samples 1 and 5 have practically the same pH, sample 5 shows a much poorer gold number which is very likely due to the fact that it is a much lower grade gelatin as is shown by the other tests and characteristics used in examining these gelatins.

*Experiment II. Comparison of gel strength.* The results obtained in experiment I with the Burke Gelatin Standardizer were carried out according to Burke's method using 5 grams of gelatin made up to 100 cc. In the previous experiment the moisture content of these six gelatins was found to vary from 10.99 to 13.85 per cent. In this experiment the work on gel strength was repeated using a true 5 per cent solution, taking into consideration the moisture content of the gelatins. The amount of gelatin required to make a true 5 per cent solution, the results obtained on the Burke Gelatin Standardizer, and the amount of each of these gelatins that should be used in the ice cream mix according to Burke's (2) (1923) table for plunger fall are found in table 2.

The gel strengths are uniformly higher in the case of the true 5 per cent gelatin solutions, but the range between the highest and lowest reading is still very narrow. The amount of gelatin to use in the mix is less when the strength is measured using the true 5 per cent solution. However, the range is much narrower than that which was found to be the correct amount to use in the mix of each of these gelatins when a comparison was made of the finished ice creams. In operating the standardizer it was found that a difference in 1°C. in the temperature made a difference of several points in the reading, however, it was found with all determinations that the range between the samples was too narrow and moreover there was no simple relationship between the amount of gelatin to use in the mix as shown by the Burke Standardizer and the amount which was found to be the proper amount by experimenting with these gelatins in the ice cream mix.

TABLE 2

*Gel strength by Burke gelatin standardizer*

| SAMPLE NUMBER | PLUNGER FALL BURKE'S 5 PER CENT SOLUTION | AMOUNT TO USE IN MIX | GELATIN REQUIRED FOR TRUE 5 PER CENT SOLUTION | PLUNGER FALL IN TRUE 5 PER CENT SOLUTION | AMOUNT TO USE IN MIX |
|---|---|---|---|---|---|
| | | *per cent* | *grams* | | *per cent* |
| 1 | 82.0 | 0.39 | 5.75 | 91.0 | 0.345 |
| 2 | 82.7 | 0.3835 | 5.61 | 85.5 | 0.3725 |
| 3 | 81.0 | 0.395 | 5.62 | 80.0 | 0.40 |
| 4 | 74.0 | 0.43 | 5.65 | 79.0 | 0.405 |
| 5 | 74.3 | 0.4285 | 5.80 | 80.5 | 0.3975 |
| 6 | 84.7 | 0.3765 | 5.64 | 88.5 | 0.3575 |

## A STUDY OF GELATINS IN ICE CREAM

Experiments III to V inclusive are devoted to a study of the gelatins in the ice cream mix, and their effect on the resulting ice creams. It was deemed advisable to study the effect of each gelatin on the viscosity of the mix, the overrun obtainable under the same condition of freezing, the retention of air by the frozen mixes, the standing up qualities or melting resistance of the finished ice cream, and the effect of the gelatins on the flavor and texture of the ice creams after being hardened. A check sample containing no gelatin was compared with the ice cream containing the gelatins in this series of experiments. Sample 7 in this part of the investigation represents the check sample and contains no gelatin.

*Experiment III. The effect of various amounts of gelatin on the viscosity of the ice cream mix.* The results obtained in experiment II in which the strengths of the gelatins were measured indicated that approximately 0.4 per cent of any of the gelatins with which the investigation was conducted would be sufficient gelatin to use in an ice cream mix. The object of this experiment was to study the effect of varying amounts of the gelatins on the viscosity of the mix. Ice cream mixes were prepared containing 0.5, 0.4, and 0.3 per cent of the gelatins and viscosity determinations were made on the fresh mixes and on the mixes after aging twenty-four hours. The average viscosity reading of three mixes in case of the 0.5 per cent gelatin ice cream, four mixes in case of the 0.4 and 0.3 per cent gelatin creams, and eleven mixes in case of the ice cream containing no gelatin are found in table 3.

TABLE 3

*Effect of gelatin on the viscosity of the ice cream mix*

| SAMPLE NUMBER | VISCOSITY OF 0.5 PER CENT GELATIN MIXES | | VISCOSITY OF 0.4 PER CENT GELATIN MIXES | | VISCOSITY OF 0.3 PER CENT GELATIN MIXES | |
|---|---|---|---|---|---|---|
| | Fresh | Aged | Fresh | Aged | Fresh | Aged |
| 1 | 1576.3 | 4523.6 | 682.6 | 1966.5 | 369.5 | 764.3 |
| 2 | 1226.7 | 3531.6 | 483.5 | 1401.8 | 389.7 | 662.6 |
| 3 | 810.9 | 2121.2 | 343.0 | 748.9 | 316.3 | 543.2 |
| 4 | 417.0 | 839.3 | 298.0 | 523.7 | 301.3 | 439.0 |
| 5 | 483.0 | 1035.8 | 279.5 | 447.6 | 373.1 | 536.5 |
| 6 | 2852.1 | Set to gel | 1504.5 | 3336.4 | 474.5 | 983.4 |
| 7 | 464.5 | 677.3 | 464.5 | 677.3 | 464.5 | 677.3 |

From the results of this table, and the visible viscosity which could be observed on pouring the mixes, it was apparent to the authors that 0.5 per cent gelatin was too much gelatin to be used in the mix in the case of gelatins 1, 2, 3, and 6. The other two gelatins resulted in an ice cream mix that increased slightly more than the check mix in viscosity on aging. When the amount of gelatin was decreased in the mix to 0.4 per cent it was found that there was a decrease in the viscosity of all the mixes, but this still appeared to be too much gelatin in the case of gelatins 1 and 6, and probably gelatin 2. This proved to be about the correct amount to use in the case of gelatin 3, and the mixes containing gelatins 4 and 5 did not increase as much in viscosity on aging as the check mix. Passing to the 0.3 per cent gelatin mixes it appeared that this amount of gelatin was still a little too much in the case of gelatin 6, and the results indicated that it would probably be better to use a little more than 0.3 per cent of gelatin when using the No. 2 gelatin. While the mixes containing gelatins 3, 4, and 5 increased slightly in viscosity on standing, this increase was less than the check mix. This experiment indicates that gelatin has a marked influence on the viscosity of the ice cream mix and that too much gelatin makes for a plastic mix that is heavy and soggy.

*Experiment IV. Retention of air by the mixes.* A common fault often found with ice cream is that it 'drops' in the can, or settles. This fault is caused by the air leaving the same, and it was the purpose of this experiment to observe the relative length of time these gelatin ice creams would retain their air at room temperature. In all these experiments the air was always out of the check sample after holding in the cold room (1°C.) over night. In the case of the mixes containing 0.5 per cent gelatin the air completely left samples containing gelatins 3, 4, 5, and check sample 7 at the end of 28 hours at room temperature. The air had just started to leave sample 1 and sample 6 at the end of this period.

In the case of the 0.4 per cent gelatin mixes samples 1 and 6 placed at room temperature had not changed at the end of 5 hours, while samples 4 and 5 had lost nearly all their air at the end of this period. At the end of twenty-four hours all of the samples except 1 and 6 had completely lost their air, and very little air remained in these samples (nos. 1 and 6) at the end of this period.

In case of the 0.3 per cent gelatin ice creams all of the samples had started to lose their air by the end of five hours.

The range in overrun in the ice cream samples of this experiment was not very wide and could not be given as a reason for the difference in retention of air. In every case the gelatin mixes retained air longer than the check sample and the more viscous mixes retained the air for a longer period. The viscosity of the mix seemed to have a marked influence on the retention of the air in the finished ice cream, the difference in viscosity being due to gelatin.

*Experiment V. Standing up qualities or melting resistance of the gelatin ice creams.* The melting resistance and the standing up qualities of the ice cream is of considerable importance from the economic side of the ice cream industry. In warm weather a few degrees difference in melting temperature is very important and it was the purpose of this experiment to determine the rate of melting of ice cream containing the various gelatins. Plate I shows the ice creams at the beginning of the experiment, and plates II and III show the 0.5 per cent gelatin ice creams after being held two and three hours, respectively, at 22°C.

At the end of three hours the check sample was nearly completely melted and the part that was left above the wire gauze was mostly foam. Ice cream containing gelatin 6 did not melt but a small amount of a light yellow liquid had come out of the mix at the end of three hours. This liquid was very sweet and did not have the characteristic ice cream taste of the liquid from the check sample. The reason why this sample did not melt may have been due to its power to imbibe large quantities of water and thus hold the water in the ice cream mix. The liquids from samples 4 and 5 had the ice cream taste but they were slower in melting than the check sample. The other four samples all became soft, and all but sample 6 spread out on the wire guaze. These mixes were leathery and gummy.

The standing up qualities of the 0.4 per cent gelatin ice creams are shown in plates IV and V. It will be noted that the check sample appears quite similar to the check sample shown in plates II and III which illustrate the 0.5 per cent gelatin ice creams, but samples 4 and 5 in the experiment melted down without leaving any foam on top of the wire gauze. Sample 6 did not melt but did break earlier and spread out more. Samples 1, 2, and 3 melted slightly, but most of the mix spread out and ran off the side of the wire gauze.

The results of the 0.3 per cent gelatin mixes are shown in plates VI and VII. Here it will be noted that samples 1, 2, 4, and 5 had completely melted at the end of three hours, and also that these mixes had melted through the wire gauze. Sample 3 was also nearly all melted but some of this sample ran over the side of the gauze. Sample 6 still refused to melt like the other samples of ice cream at this concentration of gelatin. While sample 6 did not melt at any concentration of gelatin used in this investigation, it was not an ice cream that would take with the public. It was a heavy, soggy, and leathery ice cream that was hard to dip and left an after-taste in the mouth after eating. The same relationship existed between the standing up qualities of these gelatin ice creams and viscosity as there was between the retention of air and the viscosity. The more desirable ice cream was the one that melted down smooth and the liquid had the characteristic ice cream taste.

## DISCUSSION

The practical ice cream manufacturer is interested in the grade of the gelatin and the amount of the gelatin he must use in his mix to obtain the results which he desires. In the experiments with the use of these gelatins in the ice cream mix special attention was given to this point and a study of the effect of the gelatins on the finished ice cream indicated that the proper percentage of each of these six gelatins to use in the mix to make a desirable commercial ice cream was as follows: Gelatin 1—0.3 per cent, Gelatin 2—slightly more than 0.3 per cent, Gelatin 3—0.4 per cent, Gelatin 4 and Gelatin 5—0.5 per cent, and Gelatin 6—less than 0.3 per cent. If the ice cream manufacturer assumes that 0.5 per cent is the correct percentage of gelatin to use of all lots of gelatin which he buys, he is sure to have trouble as the results of this investigation show that this percentage of some grades of gelatin results in an ice cream that is heavy, soggy, gummy, leathery, and gives the ice cream an undesirable after-taste. The use of a high grade gelatin is not to be discouraged because the same results can be obtained by using a smaller percentage of such gelatin and a very desirable ice cream can be made with it by using the proper percentage. However, it is much more important that the proper percentage be used with the high grade gelatin because of the marked effect of this gelatin on viscosity of the mix, melting resistance, body, texture, and flavor of the finished ice cream.

## CONCLUSIONS

1. No direct relation exists between the gold number of gelatin and the common tests used by the ice cream manufacturer in selecting gelatin with the possible exception of the swelling test.
2. There was no direct relation between gel strength, as measured in this paper, and the amount of gelatin to use in ice cream.
3. Gelatin has a marked influence on the viscosity of the ice cream mix but too much gelatin results in a plastic mix which is heavy and soggy.
4. Ice cream containing gelatin will retain air much longer than ice cream containing no gelatin, this property, however, varies with the amount and grade of gelatin used in the mix.
5. The following tests should be used by the ice cream manufacturer in selecting gelatin for use in ice cream:
    (1) Bacterial count.
    (2) Ash content.
    (3) Moisture content.
    (4) Swelling test.
    (5) The effect of the gelatin on the standing up property of ice cream at room temperature.

6. Until further contributions are made to the subject, the most practical method of judging gelatin for ice cream manufacture is that of actually testing the gelatin in ice cream and noting the effect on the standing up properties of the ice creams at room temperature.

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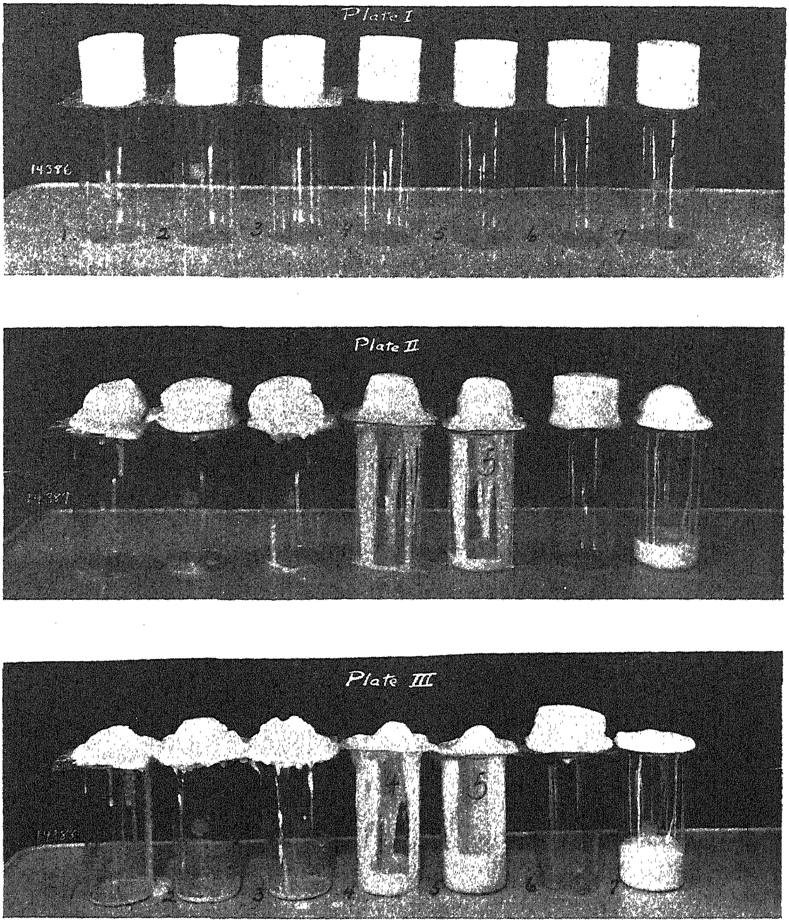
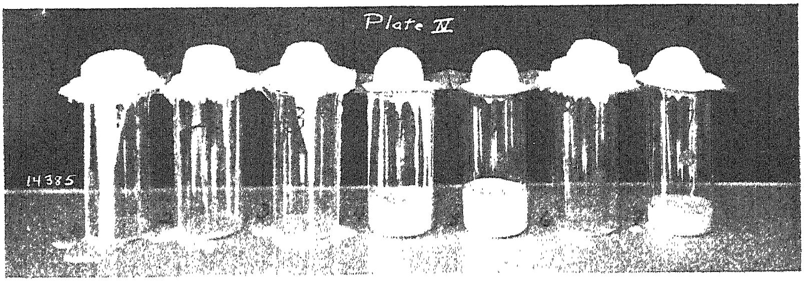
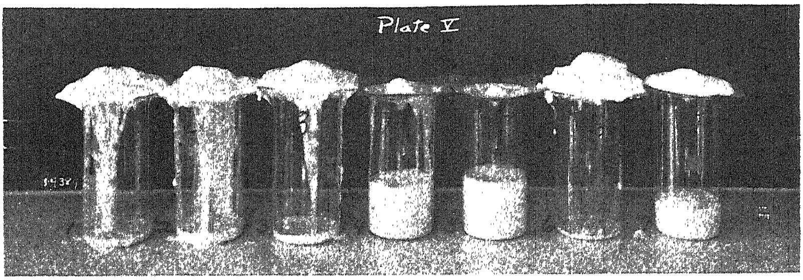


Ice Creams Containing 0.5 Per Cent of the Gelatins Studied

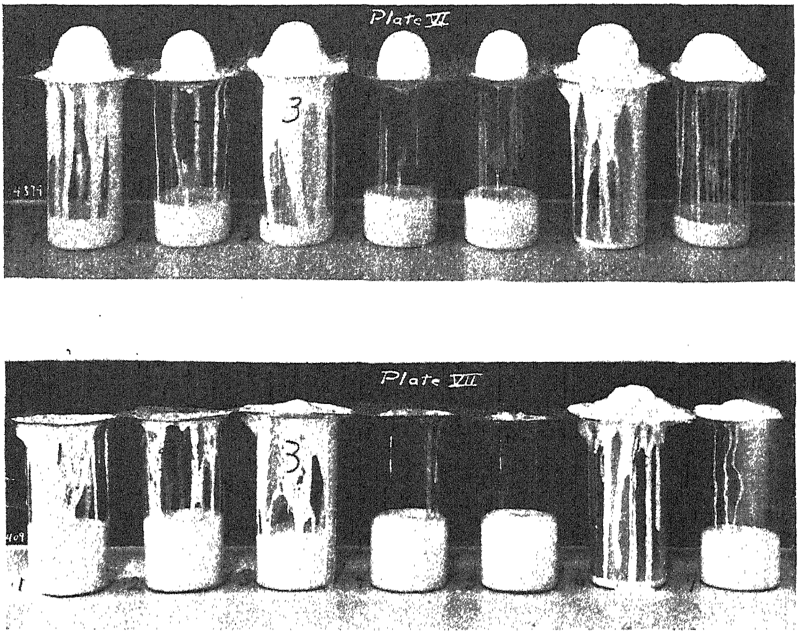
Plate I illustrates the ice creams at the beginning of the experiment, plates II and III the ice creams after standing at room temperature two and three hours respectively. Samples 1 to 6 inclusive contain the gelatins under study, sample 7 is the check containing no gelatin.





Ice Creams Containing 0.4 Per Cent of the Gelatins after Setting at Room Temperature Two and Three Hours Respectively

Samples 1 to 6 inclusive contain the gelatins under study, sample 7 is the check containing no gelatin.



Ice Creams Containing 0.3 Per Cent Gelatin Placed at Room Temperature for Two and Three Hours

Samples 1 to 6 inclusive contain the gelatins under study, sample 7 is the check containing no gelatin.

# THE EFFECT OF HOMOGENIZATION, CONDENSATION AND VARIATIONS IN THE FAT CONTENT OF A MILK UPON THE KEEPING QUALITY OF ITS MILK POWDER*

GEORGE E. HOLM, G. R. GREENBANK AND E. F. DEYSHER

*Research Laboratories, Bureau of Dairying, United States Department of Agriculture, Washington, D. C.*

There is considerable difference of opinion with respect to the relative keeping quality of milk powders manufactured by different processes. Coutts (1), also Palmer and Dahle (2), have noted that the drum process produced powders superior in keeping quality to powders manufactured by the spray processes. Palmer and Dahle have attempted to explain these results on the basis of the physical structure of the powders, showing that in a spray powder air is occluded in the milk particles and therefore the fat in this powder is subjected to the action of oxygen from within as well as upon the outer surface. These authors also suggest that homogenized fat in a powder produces a condition favorable for accelerated oxidizing action of air due to the increase surface area.

The present writers (3), though having no experiments to prove that drum powders had a better keeping quality than spray powders, accepted the observations made by the above mentioned authors, but maintained also that the quality of the fat largely determined the keeping quality of the powder manufactured.

Later experiments by Dahle and Palmer (4) do not fully substantiate their former conclusions. They maintain that a spray powder, which contains its fat in a finer state of division, and therefore exposes the greatest surface to the action of air, seems to possess the best keeping quality in this case. They also intimate that a spray powder owes its superior keeping quality to closer packing because of its fineness, and also to the smaller air cells occluded within the granules. The lack of the latter condition

* Received for publication June 25, 1925.

hardly accounts for the inferior keeping quality noted by these authors for the drum powder.

If comparisons of methods of manufacture or comparisons of products produced by different processes are to be made, it is obvious that it would be ideal if samples of the same milk could be used throughout. Where the history of the milks, i.e., age, cooling, heat treatment, fat content, mechanical treatment, etc., are identical, comparisons are justified provided the method of judging the product takes into consideration the initial stability or susceptibility of the powder to oxidation in each case.

It is also essential in comparing powders, that the moisture content (better vapor pressure of the powders) be as nearly equivalent as possible, and that they be thus maintained throughout the tests. The variability in moisture content is of especial importance where deterioration (oxidation) is judged by the olfactory sense, since it has been shown (5, 6) that free moisture has a decided effect upon the stability of the compounds responsible for the tallowy odors and flavors. A relatively dry sample is apt to show deterioration in shorter time than a sample of higher moisture content, when in fact the rate of deterioration (oxidation) may be the same.

Any experiments, therefore, where the above mentioned conditions are not carefully controlled are subject to irregular and erroneous results and sometimes erroneous conclusions.

The aim of this investigation was to determine the value of some basic methods of procedure, involved in the processes of milk powder manufacture, in improving the keeping quality of the product. The effect of condensation, homogenization or its equivalent in the process of drying and the variations in fat content of milks, upon powder produced will be discussed in this paper.

## EXPERIMENTAL

The effect of acid content of a fat upon its resistance to oxidation has been recorded in a former publication (7). It has also been noted that an aged sample of milk always produces a butter oil of lowered resistance to oxidation. For these reasons samples of milk more than twelve hours old were never used in the following experiments. Fresh samples of milk that had been properly cooled were standardized to the fat content required, heated to 60° to 65°C. for approximately one-half hour, and reduced to powdered form by the spray method. Approximately the same moisture content was maintained. The various powders were sealed in small tin cans and stored at room temperature. From time to time a can from each sample was opened and tested. The time when tallowiness first appeared, as judged by several individuals, was noted. These results are plotted in figure 1.

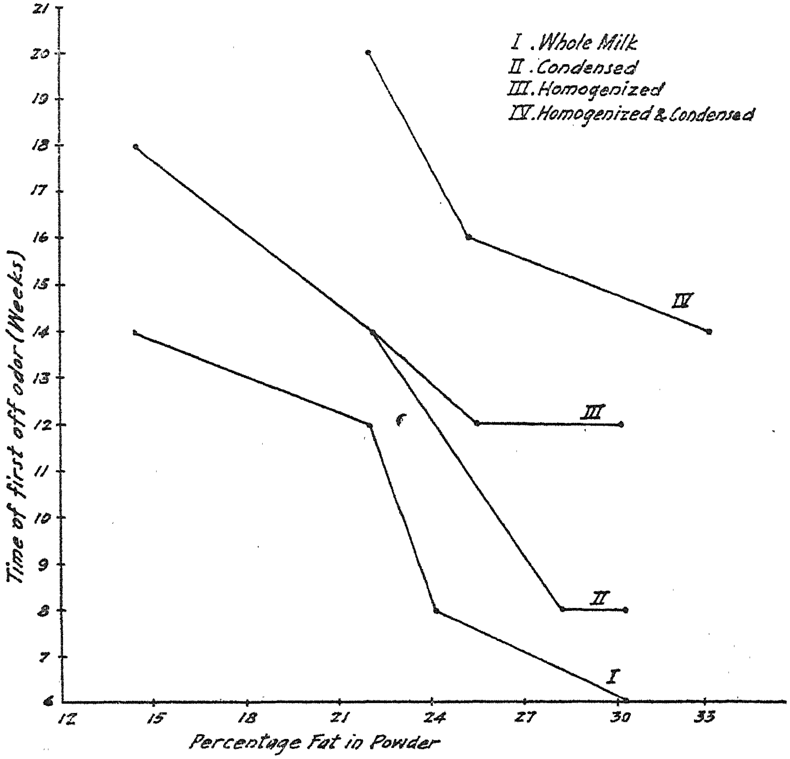


FIG. 1. SHOWING THE EFFECT OF VARIATION IN FAT CONTENT OF MILK POWDERS MANUFACTURED FROM WHOLE, CONDENSED, HOMOGENIZED, AND HOMOGENIZED AND CONDENSED MILKS UPON THEIR KEEPING QUALITY

In view of the fact that the olfactory sense does not give an entirely satisfactory measure of the actual condition or state of the fat in the powder, and gives no measure of susceptibility changes to oxidation which takes place in the fat with aging, the experiments were repeated in part.

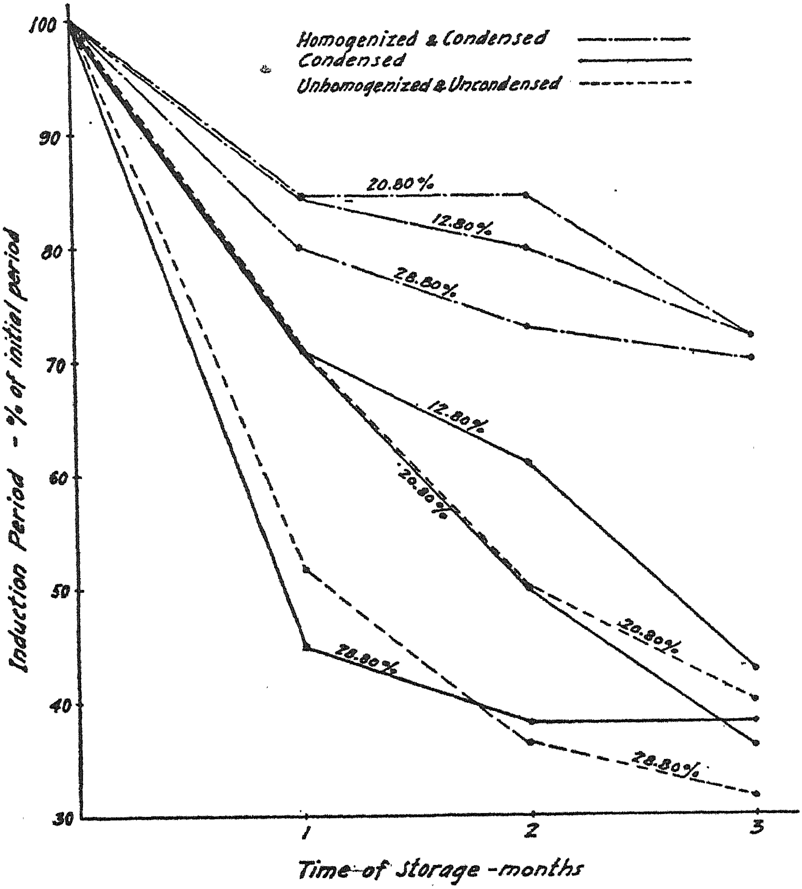


FIG. 2. SHOWING THE EFFECT OF VARIATION OF FAT CONTENT, HOMOGENIZATION, CONDENSATION, AND HOMOGENIZATION AND CONDENSATION OF A MILK UPON THE RESISTANCE OF THE FAT IN ITS POWDER TO OXIDATION

Samples were prepared and stored as described above. From time to time, the induction period, or the time necessary to cause the fat in the milk powder to absorb oxygen at 70°C., was determined by the method described by Greenbank and Holm (8). By this method it was possible to follow directly the susceptibility changes in the fat, upon storage. The initial induction period, or time necessary for oxygen absorption to begin, was determined upon each sample, thus correcting as far as possible any variation in powders manufactured from different milks. The results are plotted in figure 2.

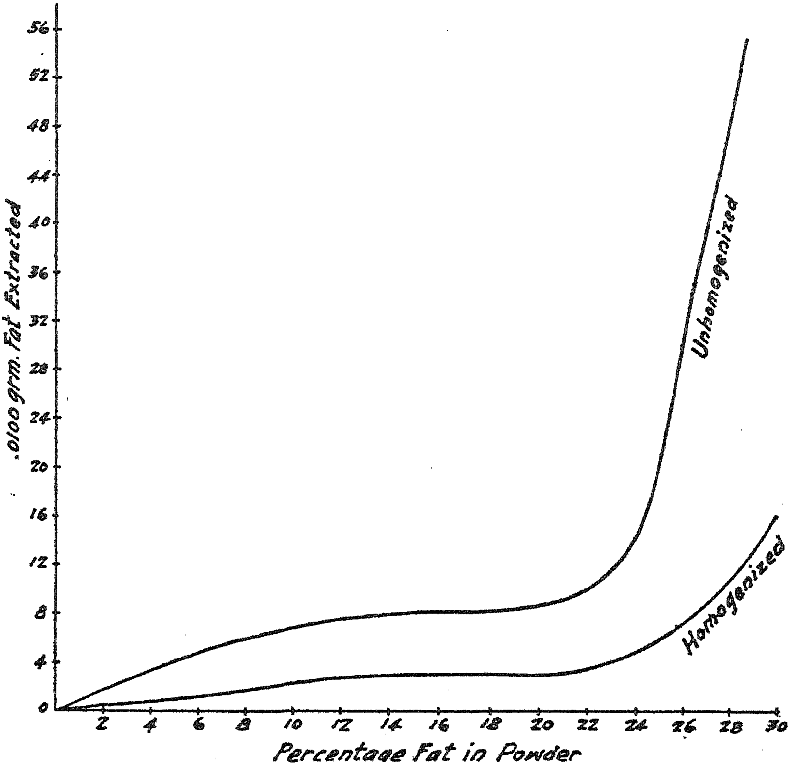


FIG. 3. SHOWING THE AMOUNTS OF FREE FAT EXTRACTED FROM MILK POWDERS OF VARYING FAT CONTENT MANUFACTURED FROM HOMOGENIZED AND UNHOMOGENIZED MILKS

The results of these two sets of experiments seem to indicate that there is quite a perceptible decrease in keeping quality with increased fat content, especially within the higher ranges. The quality of the powder seems also to be materially improved by condensation in the first set of experiments (fig. 1). These results are not so pronounced in the confirmatory experiments (fig. 2). The beneficial effect of homogenization is well illustrated in figures 1 and 2.

In some preliminary experiments it has been noted that the amount of free fat (fat not protected by a protein film) as determined by a $CCl_4$ extraction, would vary with different powders, especially in powders of higher fat content. It was decided to determine if the increase in free fat in the stored samples and their susceptibility to oxidation bore any direct relationship. Milk powders of from 20 to 30 per cent fat content manufactured from homogenized and unhomogenized milks were extracted with $CCl_4$. Varying the time of extraction and the amount of sample used showed very little effect upon the per cent of fat extracted. The results showing the variation in free fat in these powders are plotted in figure 3.

## DISCUSSION

The comparison shown in figure 1 seems to indicate that a precondensed sample of milk will produce a powder of better keeping quality than will the unprecondensed. This is in accord with the experiments carried out by the authors upon pure butter oil. In every case where a pure butter oil was treated in such a manner that some of the volatile or water soluble products were removed, the keeping quality was improved.

The extent to which different heat treatments of the milk affected the keeping qualities of its powders has not been ascertained. It is reasonable to assume, however, that any increase in heat treatment will improve the keeping quality of a powder, since the tendency of heat treatment, when applied to a reasonably fresh product, is to remove the volatile substances or in some way remove the substances which act as catalysts for the oxidation.

Homogenization seems to have a decided effect in improving the keeping quality. This is strikingly brought out in the second series of experiments, where resistance to oxidation was measured directly by the ability of the fat in the powder to withstand oxygen absorption. The mechanism of prevention of oxidation in this case is not clear.

The suggestion first made by Palmer and Dahle that the fat in a spray powder oxidizes more rapidly than does the fat in a drum powder, and that the oxidizing action is induced by a greater surface exposure due to greater subdivisions of fat globules, seems to be strongly contradicted.

Whether the protective action against oxidation given by homogenization is largely physical in nature or both physical and chemical, still remains an open question.

The antithesis of the above idea, namely, that presence of free fat in a powder should increase susceptibility, appears perhaps to furnish an index to susceptibility, other conditions being equal.

A correlation of figures 1 and 3 indicates that the breaks in the curves obtained for the keeping quality, and those obtained for free fat extracted, occur at approximately the same per cent fat content. No extracts were made upon the samples used in the second set of experiments. Though definite proof is lacking as to the exact nature of the retarding action, the results indicate that the limited covering power of a milk for the butterfat has much to do with the keeping quality of the milk powder manufactured from this milk.

It has been reported by Supplee (9) that increases in fat content from 5 per cent to 55 per cent showed progressive improvement in keeping quality. These results are in direct contradiction to the results obtained by the writers for powders of fat contents up to 33 per cent. The author does not state, however, how the fat content of the milk was increased.

Powdered cream presents an entirely new problem, in which the intensity factor of the various conditions must be considered even more carefully. Direct comparison between the fat in this product and that in whole-milk powder must be made with even more care than when whole-milk powders are compared.

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# THE ENERGY REQUIREMENTS OF DAIRY COWS*

## II. IS NET ENERGY OR METABOLIZABLE ENERGY THE MORE USEFUL INDEX FOR PRACTICAL PURPOSES?

EDWARD B. MEIGS

*Research Laboratories, Bureau of Dairying, United States Department of Agriculture, Washington, D. C.*

### INTRODUCTION

In the previous article of this series (9) it was shown that the experimental results obtained at the dairy experiment farm of the United States Department of Agriculture, at Beltsville, Md., are in close agreement with those of Haecker and Eckles in regard to the quantities of total digestible nutrients which are necessary to keep dairy cows at uniform body weight. It was shown also that the experiments of Eckles, on the one hand, and of this station on the other, which are in close agreement in regard to the quantities of total digestible nutrients necessary to maintain uniform body weight, are in less close agreement when their results are calculated in terms of net energy values; and, further, that this situation is readily explained by supposing that net energy values are a less accurate measure of the relative values of different feeds for maintaining uniform body weight than are total digestible nutrients. It is proposed in this article to inquire into the physiological justification for using either total digestible nutrients or net energy values as a measure of the practical value of feeds.

An admirable discussion of the distribution of energy in foodstuffs from the physiological point of view is given by Armsby (5, pp. 635–678). It will not be necessary to repeat it here. It need only be said that the terms developed by him will be used in this discussion in the sense in which he defined them. The gross energy of a food, according to Armsby, is the maximum amount of energy which it can furnish for the vital activities by its oxida-

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* Received for publication January 15, 1925.

tion in the body, and is measured by its heat of combustion (5, p. 635). The metabolizable energy is the difference between the chemical energy of the feed and that lost in the excreta, or is that portion of the energy of the feed which is capable of being converted into other forms of energy in the body, either during the changes which the feed undergoes in the digestive tract or in the course of metabolism in the tissues (5, pp. 639, 640). Net energy is that portion of the energy of the feed which remains after deducting the losses of energy in the excreta and those due to the increased heat production which results from the consumption of the feed (5, p. 659).

What is meant by the total digestible nutrients of a feed is so well known as to need no recapitulation here. But it is worth while to point out that the figures for total digestible nutrients as given in the text book of Henry and Morrison (7, pp. 653–666), are intended to be a measure of the metabolizable energy of the feeds concerned, and that it is for this reason that the weight of digestible fat in the feeds is multiplied by the factor 2.25 in calculating the total digestible nutrients (see Henry and Morrison, (7, pp. 39–41)).

The meaning of the term "net energy value" and the experimental method by which net energy values have been determined may be further elucidated as follows. It has been found that when food is given to a partially or completely starving animal, not all of its metabolizable energy is used to replace that of the body tissue which the animal was previously burning up. The result usually is that while the waste of the animal's body tissue is lessened, its total heat output is, at the same time, increased. In other words, only a part of the energy of the food given is used to prevent waste of body tissue; the rest is converted into heat. In Armsby's experiments the heat output of animals is determined, first during a period in which they are fed a markedly submaintenance ration, and then during a period in which they are fed a larger though still somewhat submaintenance ration of the same food material. From the data so obtained that portion of the energy of the extra food given in the second period which is turned into heat and that part which is used to save body tissue are calculated. The latter portion is called the net energy value of the food (5, p. 271).

In other cases the net energy values of foods for fattening animals have been determined. In such experiments additional food is given to an animal which is already on a maintenance or super-maintenance ration, and it is determined what proportion of the metabolizable energy of the added food is stored by the animal as body fat or other body substance and what proportion is lost as heat. That portion of the metabolizable energy of the food which is stored as body fat or other body substance is called the net energy value of the food for fattening or growth (5, p. 360).

It is proposed in this article, first to point out that the results of past experiments have been such as to indicate that it will be very difficult to obtain satisfactory average figures for the net energy values of foods, even for the very special conditions which have obtained in the calorimetric experiments. In a later section, it will be inquired whether any figures which could be obtained in such experiments are likely to have a greater value for indicating the relative usefulness of foods in practice than are figures for the metabolizable energy values of the foods in question.

## THE VARIABILITY OF THE FIGURES OBTAINED IN PAST CALORIMETRIC EXPERIMENTS

The writer has made a particularly detailed study of Armsby's work. It is to be noted, however, that the same fundamental idea underlies the work of Armsby and that of Kellner; and the same criticisms, therefore, apply in a general way to both. The following discussion is confined chiefly to Armsby's work merely for purposes of clearness and definiteness. The writer also wishes it to be understood that he has the most sincere admiration for the care and accuracy with which Armsby's experiments were carried out and for the clearness with which very difficult and complicated matters have been set forth in the published accounts of them; he fully realizes that Armsby and Kellner and their collaborators have achieved scientific advances of great permanent value. But he is none the less convinced that the limitations of the net energy conception should be fully realized by every one who is interested in the science of nutrition and the practical feeding of farm animals.

The conceptions, net energy value and starch value,[1] distinguish between that part of the energy of a food which is used to maintain or increase body substance and that part which is turned into heat. Net energy values and starch values are determined by measuring and comparing the heat output of animals in two separate periods in which they receive different amounts of food.

Now the heat output of an animal depends very largely on its muscular activity, and the extent to which a living animal uses its muscles in any given period depends on many other factors besides the food which it receives during that period. The results of an experiment to determine the net energy value of a food would be vitiated if, for example, it happened that the experimental animal were successfully protected from flies in one of the periods, but that flies gained access to it in the other period. The muscular activity and consequently increased heat output occasioned by the flies in one of the periods would introduce a very large error. Similarly, if the temperature were much lower in one of the periods than in the other, the animal might be caused to shiver; and this also would introduce a very large error.

In order to avoid errors of the kinds referred to, it is necessary to keep the animals used in this type of experiment under uniform conditions. Armsby and Fries give a striking description of the precautions which they used. There were no external noises except the monotonous click of the meter pump, no visitors were admitted, the temperature in the calorimeter was kept at from 17° to 18°C., even the change from daylight to artificial light was hardly perceptible, and the animals were all docile and accustomed to the experimental conditions (4, pp. 464–475).

A careful study of the experimental results, however, shows that in spite of these precautions the muscular activity of the animals was affected by uncontrollable influences as well as by the nature and amount of the food given. It was found, for instance, that the animals gave off about 40 per cent more heat when standing than when lying down (3, p. 23), that the proportion of time spent standing varied from 24 per cent to 100 per cent of the whole period spent in the calorimeter and was, to a large extent, independent of the food consumed (4, p. 460–462).

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[1] For a discussion of the meaning of "starch value," see Armsby (5).

It is not an easy matter to decide how to deal with this difficulty. It is obvious, of course, that an animal may be induced to spend a large proportion of its time standing by uncontrollable influences other than the food which it receives, and that large errors will be introduced into the determinations of net energy, if the standing due to these chance extraneous influences is counted as due to the influence of the food. On the other hand, there is no doubt that an increased food supply has a general tendency to cause increased muscular activity in animals, and this tendency appears quite clearly in the results of Armsby's experiments. It was found, for instance, that the animals which received more food had a decidedly greater tendency to change often from the standing to the lying position, though only a slight tendency to spend a larger proportion of their time standing (4, p. 460). In order to obtain really accurate results, therefore, the investigation would have to distinguish between the muscular activity due to accidental extraneous influences and that due to increased food intake.

Armsby and Fries apparently changed their opinion in regard to the question whether the changes in heat output due to standing and lying down should be counted as due to changes in food intake or regarded as accidental. In Bulletin 51 of the Bureau of Animal Industry (1) they take this factor into account in drawing conclusions from their results. In Bulletin 74 of the same series (2), on the other hand, they calculate the net energy values of the foods used without considering what proportion of the time the animals spent standing or lying in the various periods of the experiments. In Bulletin 101 of the same series (3) and in later articles, they return to the practice of taking into account the proportion of time spent standing.

Whichever way the results are calculated, they vary widely in different individual experiments. In Bulletin 74 of the Bureau of Animal Industry, for instance, the results, calculated without regard to the proportion of time which the experimental animal spent standing or lying in the different periods, lead to the conclusion (2, p. 40) that the net energy of clover hay amounts to 36.42 per cent of its metabolizable energy. In Bulletin 101, the authors correct the results to the supposition that the experimental animal spent 17 hours standing and 7 hours lying down each day. From the figures given on pages 32 to 35 (3), however, it is possible to calculate the results on the same basis as in Bulletin 74 (2); when this is done, it appears that in the two most satisfactory experiments,[2] the net energy of the clover hay was about 85 and 64 per cent of its metabolizable energy respectively. Correcting the results to the supposition that the ox spent seven hours of each twenty-four lying down, the authors find in three different cases that the net energy of the hay was 79.99, 72.90, and 92.74 per cent of the metabolizable energy respectively (see 3, p. 40).

Further data on the distribution of nutritive energy in clover hay are given in a still later article (4, p. 473). In this case, the increments in heat production caused by clover hay per kilogram of dry matter are given, the results being corrected to the supposition that the experimental animals spent half their time lying down. It appears that in one case the hay caused the heat output to be increased at the rate of 992 calories per kilogram of dry matter fed; in another case, at the rate of 453 calories per kilogram. In order to compare these results with those given above, it is necessary to know the metabolizable energy of clover hay. This quantity is comparatively constant; in figures given on page 34 of Bulletin 74 and on page 32 of Bulletin 101 the utmost variation is from 2.290 to 2.024 calories per gram of total organic matter in the hay, and the average may be taken as approximately 2040 calories per kilogram of total dry matter.[3] Using this figure for metabolizable energy, the figures given above would mean that in one experiment the net energy was 51 per cent of the metabolizable energy; in the other, 78 per cent. These figures are to be compared with those of Bulletin 101, in which the authors calculate that the net energy of clover hay is from 72.90 to 92.74 per cent of the metabolizable energy.

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[2] Some of the experiments described in this bulletin are vitiated by the fact that the ox, for unknown reasons, stood up during the whole 48 hours of one of the periods.

[3] About 6 per cent of the total dry matter of clover hay is ash, and therefore the metabolizable energy per kilogram of total dry matter is somewhat lower than the energy per kilogram of total organic matter.

The figures given by Armsby and Fries for the net energy of clover hay are perhaps somewhat more variable than those given for other foods. But the other figures are also highly variable. On page 473 of the article in the Journal of Agricultural Research above cited, for instance, are given figures for the increments in heat production per kilogram of dry matter caused by timothy hay. These range from 529 calories to 954 calories. It is a very moderate statement, therefore, to say that the net energy of any given feed may often turn out to be 50 per cent more in one experiment than it is in another, even when the results are calculated with all the refinements and precautions described by Armsby and Fries in the article just cited.

This variability in the results is not in the least surprising; on the contrary, it is entirely to be expected. The reasons for it have already been outlined and may be restated as follows: The heat output of an animal, under such conditions as those that obtain in the experiments of Armsby and Fries, is largely dependent on the degree of its muscular activity, and the muscular activity is influenced, not only by the food intake, but also by other accidental factors which it is impossible to control. When Armsby and Fries correct for the proportion of time which their animals spend standing, they tacitly admit that one form of muscular activity may be accidentally increased in such a way as to interfere with the value of the results. But the muscular activity involved in standing is only one form; another is that involved in frequent changes from the lying to the standing position; still another expresses itself in increased restlessness either while standing or while lying down. Armsby and Fries make no attempt to correct for these other forms of muscular activity; and, as a matter of fact, it is impossible to correct satisfactorily either for them or for the muscular activity involved in standing. For there is every reason to believe that increased food intake stimulates to increased muscular activity, and whatever part of the muscular activity is so caused must be taken into account as a part of the effect of the food. But, in order to get ideal results, this part of the muscular activity would have to be distinguished from the part which was accidental, and to make this distinction is impossible in such experiments as those under consideration.

The writer has made a careful study of the experimental basis for some of the net energy values published in Armsby's book (5, pp. 715–721) and has compared these figures with figures given by Kellner (8, pp. 627–639) for the starch values of the same foods. The experimental results on which the figures given in the book rest are quite variable and not very numerous, and the net energy values do not run at all parallel to the starch values for the same foods. It is, however, not necessary to go into this question in detail, as it is generally realized that the determinations of net energy value were incomplete at the time of Dr. Armsby's death. In a recent note (6), his successor has stated that all the figures for net energy values which have been published from the Institute of Animal Nutrition of the Pennsylvania State College are to be recomputed and corrected, and that Dr. Armsby himself regarded the published results as tentative.

## WOULD IDEAL FIGURES FOR NET ENERGY AS DETERMINED IN ARMSBY'S EXPERIMENTS BE A BETTER INDICATION OF THE RELATIVE VALUES OF DIFFERENT FOODS UNDER PRACTICAL CONDITIONS THAN WOULD FIGURES FOR THEIR METABOLIZABLE ENERGY?

The experiments of Armsby and Fries, on which many of the figures for the net energy values of foods given in Armsby's book are based, were carried out under very special conditions. The animals were kept as quiet as possible in a calorimeter at a temperature of 17° to 18°C. It is legitimate to inquire whether the relative tendencies of different foods to stimulate the output of animal heat would run parallel under such conditions to their tendencies to stimulate heat output under the quite different conditions which obtain in practice.

It was the old idea that a considerable amount of energy was necessary for the mastication, digestion, and assimilation of foods, particularly of roughages; and that the heat output caused by the consumption of food in such experiments as those of Armsby and Kellner was a measure of this energy. If this idea were correct, the net energy and starch values would probably be the best possible indication of the practical usefulness of the nutritive energy contained in different foods; for it would be entirely logical to believe that the mastication, digestion, and assimilation of a given quantity of any particular food would always require about the same amount of energy, whether the animal were resting or active, and whatever the external temperature might be.

But Armsby and Fries make it quite clear that the heat output of the animals in their experiments which was caused by the consumption of food was not entirely or even chiefly a measure of the energy which was required for mastication, digestion, and assimilation. The reasons for discarding this older view are very fully and clearly given in their article in the Journal of Agricultural Research (4). On page 459 of that article, for instance, it is stated that there was frequently no increase in heat output by the animals while they were masticating their food; and rather frequently, a distinct fall. This interesting observation may be interpreted in various ways; a not improbable interpretation is that the effect of mastication was to reduce other muscular movements, so that the loss of energy through this process was compensated or overcompensated, and may be regarded as often not occurring at all from a practical point of view. But, however the observation is interpreted, it shows clearly that the increased heat output due to mastication is either zero or at most a small fraction of the total nutritive energy of the food.

On page 460 the authors give reasons for thinking that their animals were more restless on the heavier rations, and on page 479 they state that from 3 to as much as 30 or 40 per cent of the increased heat output caused by the consumption of food appears to have been due to increased muscular activity while standing. On page 477 they show that coarse woody feeds cause less increase in heat output per kilogram dry matter consumed than do concentrates. On page 479 they speak of the stimulating action which food has on general metabolism in addition to its effect in stimulating muscular activity, and express the opinion that this factor is not inconsiderable.

From the above cited passages it is fair to conclude that Armsby and Fries are strongly of the opinion that the increased heat output caused in animals by the consumption of food is, to a large extent, due to the stimulating action which food has on muscular activity and on general metabolism, and to a small extent only, to the work required for mastication, digestion, and assimilation. With this conclusion the present writer is in entire agreement. But, if this conclusion is correct, a large part of the basis for believing that the net energy and starch values of foods as determined in Armsby's and Kellner's experiments are a good indication of their practical usefulness for maintenance and fattening, is lost.

It is natural to suppose, as stated above, that the energy required for the mastication, digestion, and assimilation of a given quantity of any particular food would be the same, whether it were fed by itself or along with other foods, whether the animal which consumed it were active or resting, and whatever the external temperature might be. But it is by no means equally clear that the relative tendencies of different foods to stimulate muscular activity and increased general metabolism would remain uniform under all these different conditions. Unless this point is established, the figures for net energy values obtained in calorimetric experiments cannot be regarded as necessarily valid for practical conditions.

In the preceding paper of this series (9) evidence was presented which indicates that the figures for total digestible nutrients given in the tables of Henry and Morrison are a reasonably satisfactory measure of the relative values of foods for maintaining uniform body weight in dairy cows. These figures aim at giving the metabolizable energy of foods. But they are not an exact measure of metabolizable energy. The relation between the total digestible nutrients and the metabolizable energy of foods is clearly discussed by Armsby; he recognizes that the former is an approximate measure of the latter, and gives figures from which an idea may be formed as to how far the two sets of values may depart from strict parallelism (5, pp. 635–650). The reasons for the non-existence of strict parallelism are, in brief, that certain metabolic products, particularly methane, have not been taken account of in determining the coefficients of digestibility of the various farm foods. Armsby gives methods by which metabolizable energy may be calculated from the figures for the digestible nutrients (5, p. 650).

In order to obtain the therms of metabolizable energy in a pound of roughage, the total digestible organic matter[4] therein contained is multiplied by 1.588; the corresponding factor for grains and similar material with less than 5 per cent digestible fat is 1.769; for grains with more than 5 per cent fat, 1.814; for oil meals and materials high in protein, 2.086. The figure 2.086 is about 31 per cent higher than 1.588. This means that the extreme error that could be made by taking the total digestible organic matter of foods as a measure of their metabolizable energy would be 31 per cent.[5] Such an error, however, would be made only in cases where one set of animals was fed entirely on roughage; and the other set entirely on oil meals and materials high in protein. As a combination of roughage and concentrates is fed under almost all practical and experimental conditions, the error under consideration would be much smaller than this in most cases, and would probably seldom rise above 10 per cent.

It may be concluded, therefore, that experimental results which indicate that the total digestible nutrients are a good index of the practical value of foods as sources of nutritive energy, indicate also that metabolizable energy is a good index in the same sense. The experimental evidence obtained from dairy cows and bearing on this question has already been discussed (9). The remarkable agreement between the results of Haecker and Eckles and those obtained at the Beltsville farm in regard to the quantities of total digestible nutrients necessary to maintain dairy cows at uniform body weight indicates that either total digestible nutrients or metabolizable energy would constitute a better index of the practical value of feeds as sources of nutritive energy for this class of farm animals than any other that exists at the present time.

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[4] The total digestible organic matter contained in a food is the same as the total digestible nutrients, except that in the former case the actual weight of the fat is added to that of the other constituents, instead of the weight of the fat multiplied by 2.25.

[5] The error would obviously be somewhat less in most cases if the total digestible nutrients were taken instead of the total digestible organic matter, as the high nutritive energy of fat is allowed for in computing the former figures.

From the existing information the point should be emphasized, however, that no system of measuring the nutritive energy of foods will give more than very limited information regarding their relative practical values in a general sense. Even for animals that are simply being maintained at uniform body weight or fattened, certain definite chemical compounds must be supplied in the food; and, in the case of animals which are growing or producing milk, eggs, or wool, the problem of chemical requirements rather overshadows that of the energy requirements. Unless the chemical requirements are adequately met, the most exact system of determining the nutritive energy of different foods will be useless.

## SUMMARY

The net energy or starch value of a food is that fraction of its metabolizable energy which an animal can use for maintenance or fattening. But the value of this fraction in any particular instance depends on the heat output of the animal in question, and this again depends on the degree of muscular activity, and on the external temperature. Net energy and starch values will not, therefore, be constant for any given food unless they are determined under fixed conditions. As the fixed conditions for their experiments, Armsby and Fries have chosen an external temperature of about 17°C. and the least possible stimulation to muscular activity.

A careful study of their results shows that even under the most uniform conditions which they were able to obtain, the muscular activity of their animals was unevenly influenced by uncontrollable accidental causes, so that the results of individual experiments on the same food were highly variable. It is questionable whether their experiments were sufficiently extended to establish reliable approximate averages even for the conditions above described.

That values obtained under these conditions would not be applicable under all practical conditions is obvious. In Armsby's system energy converted into fat is regarded as net, and energy transformed into muscular activity is counted as waste; for draft animals the reverse is the case.

It is probable that net energy values obtained under the conditions of Armsby's experiments would be more or less inapplicable under almost all practical conditions. In order to suppose that they would be applicable, one must make a number of questionable assumptions, among which the most important are that the relative tendencies of different foods to stimulate useless muscular activity and other useless forms of heat output would remain constant, whether these foods were fed by themselves or along with others, and to whatever conditions of rest and activity or of external temperature the animals concerned were subjected.

Experiments carried out on dairy cows indicate that the existing figures for the total digestible nutrients of dairy feeds furnish a very good measure of the relative values of these feeds as sources of nutritive energy under practical conditions. As the figures for total digestible nutrients are the nearest existing approach to the measure of the metabolizable energy contained in feeds, it may be supposed that figures for metabolizable energy would also furnish a good system for measuring feeds as sources of nutritive energy under practical conditions.

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# DAIRY NOTES

## THE EFFECT OF INJURY ON THE COMPOSITION OF COW'S MILK

A registered Ayrshire cow in the University of Illinois herd became ruptured, leaving a swelling about 6 to 8 inches in diameter, a little in front and to the right of the udder. It was thought worth while to obtain a sample of milk from her to see if the injury might influence its composition. Analyses of samples taken from her earlier in the same period of lactation, and throughout the previous period of lactation were available for comparison. The analyses of the last two samples before the injury are given, as they are typical of the milk normally obtained from the cow. The two samples consisted of three-day composites, one taken at about five weeks and the other at about two and one-half weeks prior to the injury. The sample taken after the injury consisted of a composite made from the evening milking on the day of the injury, and the morning milking on the day following. Since the injury became so severe that recovery was not expected, the cow was killed before further samples were taken.

The analytical data are as follows:

| | TOTAL SOLIDS | ASH | FAT (ROESE-GOTTLIEB) | TOTAL PROTEIN | LACTOSE (OPTICAL) | SPECIFIC GRAVITY |
|---|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | |
| Five weeks before injury........ | 11.92 | 0.77 | 3.96 | 3.56 | 3.77 | 1.0286 |
| Two and one-half weeks before injury........................ | 11.55 | 0.64 | 4.00 | 3.13 | 3.72 | 1.0289 |
| After injury.................... | 8.86 | 0.67 | 2.44 | 3.15 | 2.50 | 1.0244 |

The table shows that after injury there was a very decided drop in the percentage of total solids, fat and lactose, and in the specific gravity. There was practically no change in the percentage of ash or total protein. The differences in composition are ascribed to the effects of the injury.

The change in the percentage content of lactose is in agreement with unpublished data obtained in this laboratory which indicate that the lactose of milk is especially sensitive to physiological disturbances.

O. R. Overman and F. P. Sanmann,
*Department of Dairy Husbandry, University of Illinois, Urbana, Illinois.*

## ERRATA

In the May issue of the Journal of Dairy Science, in the article by Messrs. R. F. Hunwicke and H. Jephcott, "The Destruction of Bacteria in the Roller Process of Milk Drying," the quoted paragraph at the top of page 208 should be inserted just after the word "summary," line 32, page 211. The paragraph in question is part of the quotation from Delepine's report.

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